THE AMERICAN JOURNAL OF Clinical Nutrition

JULY-AUGUST 1958 VOLUME 6, NUMBER 4

Editorials

Hypervitaminosis A: Its Broadening Spectrum

THE AMERICAN medical literature concerned with the clinical aspects of vitamin A appears to have completed a full turn from concern with vitamin A deficiency in humans solely to papers predominantly related to hypervitaminosis A. In fact, if one excludes vitamin A deficiency produced secondarily by some disease process of the pancreas, intestine, etc., more instances of hypervitaminosis A seem to have been reported in the past 12 years than severe forms of vitamin A deficiency.

General improvement in the American diet, a better understanding of nutrition generally, and widely increased use of potent and readily available vitamin preparations, have made severe primary vitamin A deficiency an unusual disorder in this country. The circumstances for taking excess amounts of vitamin A, the frequent delay in clinical recognition, instances of multiple cases in one report, and circumscribed geographic areas of case location all indicate that the disorder is more common than reported. These factors strongly suggest that the laity, and for that matter, many physicians, are not fully aware that excess ingestion of vitamin A in very large doses for a short period (days), or in lesser excess amounts for longer periods (weeks to months) may produce toxic effects, not only in infants, but in older children and adults.

Careful studies of patients with vitamin A overdose have been reported with increasing frequency, and with such consistency of findings, that several well-defined and readily recognizable clinical syndromes have been delineated which can be classified, as suggested by Knudson and Rothman[1] into acute and chronic types in both infants and adults.

Recent reviews of the clinical aspects of hypervitaminosis A by Oliver,[2] Breslau,[3] and Gerber, Raab, and Sobel[4] and others; of the biochemical and pathologic aspects by Nieman and Klein Obbink;[5] inclusion of discussions of this disorder for the first time in recent editions of general textbooks; the detailed discussion of it in a monograph on vitamin A by Moore,[6] and editorial comment[3,7] all attest to the growing importance of this subject. Reference to these sources will be useful for those not familiar with the clinical recognition of the syndrome.

CHRONIC HYPERVITAMINOSIS A

Following Joseph's original description of chronic hypervitaminosis A in a three-year-old male in 1944,[8] 36 well-documented cases, mostly infants and young children, plus six adults, have been reported.[2] The onset is insidious, following a variable latent period of weeks to many months (average 10 months) of daily ingestion of vitamin A in doses of the magnitude of 100,000 units (occasionally more, rarely less). The correct diagnosis was often not made for long periods after onset of symptoms.

Infants and young children with this disorder insidiously and commonly develop constitutional symptoms, anorexia, dry, itchy skin (often followed by a seborrhea-like rash or desquamation), alopecia and sparse, coarse hair, angular fissures or cracking of the lips and a palpable hepatomegaly.[2] Particularly constant and characteristic, after a latent period, are painful swellings of the extremities over such bones as the tibia, fibula, clavicles, ulna and metatarsals (areas not well protected by muscle mass) which may produce a protective limp, refusal to stand, or disturbed sleep. The characteristic subperiosteal new bone growth and cortical thickening of the involved bones responsible have been ably described by Caffey.[9] Of diagnostic help and interest is the frequently elevated blood alkaline phosphatase value and the almost invariable normal calcium and phosphorus serum level. There may be constipation, weight loss, and rarely a peculiar craving for butter.

In adults, the clinical picture differs in the greater severity of the alopecia, skin involvement, and nail changes contrasted with the relative mildness of bone and joint pain, rarity of radiographic bone changes, relatively normal blood alkaline phosphate values and lesser size and incidence of hepatomegaly. Unique in adults have been exophthalmos, menstrual disturbances, and skin pigmentation.

Some cases, in both children and adults, may manifest clinical evidence of increased spinal fluid pressure which will be commented on elsewhere. Hemorrhagic phenomena, common in experimental animals, are rare in humans except for occasional epistaxis.

The rapid and complete recovery following treatment, which consists simply of cessation of the excessive vitamin A intake is particularly impressive and diagnostically significant. The effects are manifest within three to ten days for such features as anorexia, bone pain, headache, limps, pruritus, and weight loss, and somewhat longer for others due to tissue changes.[3] Permanent sequelae are unusual.

In infants and children the clinical picture may be confused with infantile cortical hyperostosis (Caffey's Disease), acute rheumatic fever, scurvy, rickets, arthritis; and in adults, with some of these and hypothyroidism or Addison's disease.

In both children and adults, the features of avitaminosis A may be confusingly similar to hypervitaminosis A.

ACUTE HYPERVITAMINOSIS A

The acute form of the disease in infants as described in 1951 by Marie and Sée,[10] and adult cases represented by many examples in arctic explorers, presents primarily with central nervous system manifestations due primarily to an abrupt and marked elevation in spinal fluid pressure. This is evident within hours after ingestion of a single large dose of vitamin A in the magnitude of 300,000 units for infants, and of one to several million units for adults.

In infants, the clinical picture (known as the Marie-Sée syndrome) is one of acute hydrocephalus, with marked bulging of the fontanelles (often as mushroom-like protuberances on the child's head), accompanied by vomiting and agitation or drowsiness, but without evidence of meningeal irritation or focal neurologic signs. Vitamin A is absent from the spinal fluid but blood levels are elevated. This is followed by a delayed but rough correlation of the increase in spinal fluid pressure to the blood level.

Reports of adults with the acute form emanate chiefly from arctic regions and are related to ingestion of polar-bear liver, an edible portion of which may contain several million units of vitamin A. Similar effects have also been noted from a comparable single dose of medicinal vitamin A.

The symptom complex occurs within hours after ingestion, manifested chiefly by a violent headache, accompanied often by nausea, vomiting, drowsiness, etc., all supposedly related to a marked increase in spinal fluid pressure. In addition, many of those afflicted noted desquamation of the skin either generalized or localized about the lips or to the exposed surfaces, evident on the second day.

In acute intoxication, manifestations appear to result from direct toxic effects on specific tissues, and are not dependent on supersaturation of liver stores.[3] It is of interest that some

patients manifest features of both the acute and chronic form.

Laboratory studies indicate that the blood vitamin A level is markedly elevated in all chronic cases thus studied, with variations from a normal fasting range of 50 to 150 i.u. per 100 ml of serum to values most frequently ranging from 800 to 2,000 i.u. per 100 ml, with a record high of 6,660 i.u. per 100 ml. Elevated values were also noted in the acute form. This determination is, therefore, diagnostically very helpful.

One study[4] indicated that the elevated vitamin A level was predominantly in the alcohol rather than ester form, a point of possible diagnostic value as indicated by the more rapid drop in this fraction as compared to the ester form.

The severity of most chronic cases on record in the literature, coupled with the long delay in diagnosis following onset of symptoms, indicates a low clinical suspicion for this disorder and suggests that milder (subclinical) forms may exist which are not recognized at all. Few physicians query patients on excess ingestion of vitamins compared to a possible deficient intake. Blood-vitamin A determinations are not readily available.

In one report, unexplained headache in two patients with a slightly elevated blood-vitamin A level, taking moderate excess daily doses of vitamin A, cleared when its ingestion was stopped. These may be representative of the subclinical form which, unfortunately, has not been delineated.

Suspicion of mild hypervitaminosis A may be warranted in instances where dryness, itching or desquamation of skin, fissured lips, chronic headache, vague bone and joint pains, constipation, anorexia or elevated alkaline phosphatase in various combinations are chronically present and unexplained. An elevation of blood-vitamin A level, history of excess intake, bone changes by roentgenogram and prompt cessation of these clinical phenomena with a low vitamin A intake would confirm the diagnosis. A careful study of this type would be particularly helpful. The minimal aspects of the clinical spectrum of hypervitaminosis A is hazy and poorly delineated in contrast to the sharp definition of the severe forms.

The clinical picture of hypervitaminosis A rests not only on the consistency with which certain signs and symptoms have been present in published material, but by its reproduction experimentally in humans. Experimental studies of hypervitaminosis A in animals are too well documented to require further comment.

In humans, experimental studies have taken two forms. In several reported instances of human hypervitaminosis A,[3] many of the clinical features which disappeared when excessive vitamin A intake was stopped, promptly reappeared when the excessive doses of vitamin A were again administered. The blood-vitamin A level, after an initial sharp fall, tapers off more slowly for many weeks. Prompt reacerbation of symptoms is apparently possible if excess vitamin A is further ingested before the saturated liver stores are depleted. This holds true with the use of pure vitamin A as well as fish liver oil concentrates, indicating the excessive intake of vitamin A as the specific cause of the clinical phenomena.[11]

Even more important is the recent report by Hillman,[12] in which hypervitaminosis A was induced experimentally, on two separate occasions, in a healthy human male, age 40, given excess quantities of this vitamin during two periods; one of 14 days' duration; the other of 25 days' duration (spaced many months apart). The dose was in the magnitude of 1,000,000 units per day. Striking rises in blood-vitamin A levels were noted rather promptly during each test period accompanied by such clinical features as severe headache, skin changes (dryness, rash, desquamation, itching), various constitutional symptoms, polyarthralgia and bone pain, etc., in variable sequence and combinations, duplicating many of the important clinical phenomena of both the acute and chronic forms of hypervitaminosis A documented in published case reports. This important work is worth reading in the original.

A particularly unique and intriguing manifestation of human hypervitaminosis A, more common in the acute than the chronic form, is a striking increase in cerebro-spinal fluid pressure. This subject was recently reviewed

by Bass.[13] All the more puzzling have been reports that this same phenomenon may result from avitaminosis A in humans,[14,15] and that either hypervitaminosis A or avitaminosis A during early pregnancy in rats may produce hydrocephalus in the fetal offspring.

The mechanism is unknown, but excess formation of spinal fluid has been suggested. Vitamin A does not enter the spinal fluid which has been normal in cytologic and chemical characteristics, but under high pressure, as indicated by manometric readings. Equally mysterious are indications of a similar increase in spinal fluid pressure at times with use of certain of the tetracycline antibiotics in children.[16] Investigation of the mechanism of the acute hydrocephalus in these several conditions appears highly desirable with particular attention to whether a common mechanism is responsible.

The acute hydrocephalus has been manifest clinically by bulging fontanelles in infants, elevated cerebro-spinal fluid pressure readings, occasionally as papilledema, and rarely by enlargement of the head. It could explain the headache, vomiting, stupor, and vertigo noted with acute poisoning in infants from excess intake of vitamin A preparations; and in adults acutely ill from ingesting livers of certain fish and arctic mammals (particularly polar bears) known to contain millions of units of vitamin A in a consumed portion. Internal hydrocephalus could explain the exophthalmos noted in experimental animals and (rarely) in humans.[3]

Published reports indicate a paucity of localizing neurologic signs and suggest that the process is generalized. The acute hydrocephalus rapidly subsides with cessation of the excess intake, or with drainage via lumbar puncture.

The implication of hypervitaminosis A was greatly broadened when Cohlan,[17,18] successfully demonstrated the frequent production of congenital anomalies in baby rats whose mothers received excessive doses of vitamin A during the early phase of pregnancy. The reader is referred to recent reviews[3,6] for further comment and references to several confirmatory studies. Cohlan's experiments indicate that the congenital defects noted in offspring in rat litters are dependent on the days of gestation during which the overload with vitamin A is performed.[18] From the fifth to the eighth day, the vitamin overload commonly terminated pregnancy with fetus resorption, but with anencephaly in a small percentage of the survivors. From the eighth to the tenth day, anencephaly was noted in 53 per cent of the offspring: spina bifida, cleft palate, microphthalmia or anophthalmia were noted to a lesser extent. From the 11th to the 13th day, 92 per cent exhibited cleft palate and occasionally cataracts. Between the 14th and 16th day, cleft palate was observed in 49 per cent with more manifesting cataract. From the 18th to the 20th day, cataracts were the main defect. Rarely, other anomalies such as hare lip, macroglossia and other eye defects were noted. Fetal hydrocephalus was noted and has been commented upon elsewhere.

It is well to recall that maternal avitaminosis A in rats, in early pregnancy, has similar teratogenic effects, often resulting in birth of young with anomalies of the brain, its calvaria, and production of congenital hydrocephalus. Apparently both avitaminosis A and hypervitaminosis A are hazardous in the early rat pregnancy.

Recently, Millen and Woollam[19] reported that cortisone administered during early pregnancy potentiated the teratogenic effect of maternal hypervitaminosis A in rats as regards gross malformations of the brain and calvaria; and in another study[20] they demonstrated a striking increased incidence of cleft palate with this combination. It was suggested that cortisone acts by increasing the sensitivity of the developing tissues which enhanced the teratogenic effect of hypervitaminosis A.

There is no available information as to whether hypervitaminosis A exerts a similar teratogenic effect in human pregnancy, but the subject is one worthy of critical investigation. It also is not known whether cortisone has a similar potentiating effect.

The general caution about excess use of vitamin A in patients may well deserve double emphasis when pregnancy exists. The steadily increasing literature on hypervitaminosis A indicates an established wide spectrum of its

effects and carries the implication of still further broadening.

—HAROLD JEGHERS, M.D.
HOWARD MARRARO, M.D.
Department of Medicine,
Seton Hall College of
Medicine and Dentistry,
Jersey City, N. J.

REFERENCES

1. KNUDSON, A. G. and ROTHMAN, P. E.: Hypervitaminosis A: A review, with a discussion of vitamin A. *Am. J. Dis. Child.* 85: 316, 1953.
2. OLIVER, T. K., JR.: Chronic vitamin A intoxication: Report of a case in an older child and review of the literature. *A.M.A. J. Dis. Child.* 95: 57, 1958.
3. BRESLAU, R. G.: Hypervitaminosis A: Clinical review. *Arch. Pediat.* 74: 139, 178, 1957.
4. GERBER, A., RAAB, A. P., and SOBEL, A. E.: Vitamin A poisoning in adults: Description of a case. *Am. J. Med.* 16: 729, 1954.
5. NIEMAN, C. and KLEIN OBBINK, H. J.: The biochemistry and pathology of hypervitaminosis A. *Vit. and Hormones* 12: 69, 1954.
6. MOORE, I.: *Vitamin A.* Elsevier, Houston, 1957, p. 645.
7. EDITORIAL: Excessive intake of vitamin A. *Nutr. Rev.* 12: 268, 1954.
8. JOSEPH, H. W.: Hypervitaminosis A and carotinemia. *Am. J. Dis. Child.* 67: 33, 1944.
9. CAFFEY, J.: Chronic poisoning due to excess of vitamin A: Description of the clinical and roentgenological manifestations in seven infants and young children. *Pediatrics* 5: 672, 1950.
10. MARIE, J. and SÉE, G.: Acute hypervitaminosis of the infant: Its clinical manifestation with benign acute hydrocephalus and pronounced bulge of fontanel: A clinical and biologic study. *A.M.A. J. Dis. Child.* 87: 731, 1954.
11. TOOMEY, J. A. and MORISSETTE, R. A.: Hypervitaminosis A. *Am. J. Dis. Child.* 73: 473, 1947.
12. HILLMAN, R. W.: Hypervitaminosis A: Experimental induction in the human subject. AM. J CLIN. NUTRITION 4: 603, 1956.
13. BASS, M. H.: The relation of vitamin A to cerebrospinal fluid pressure: A review. *J. Mt. Sinai Hosp.* 24: 713, 1957.
14. CORNFELD, D. and COOKE, R.: Vitamin A deficiency: Case report (unusual manifestations in a 5½ month old baby). *Pediatrics* 10: 33, 1952.
15. BASS, M. H. and CAPLAN, J.: Vitamin A deficiency in infancy. *J. Pediat.* 47: 690, 1955.
16. GELLIS, S.: Editorial comment; in *Year Book of Pediatrics.* Year Book Publishers, Chicago, 1956, p. 40.
17. COHLAN, S. O.: Excessive intake of vitamin A as cause of congenital anomalies in rats. *Science* 117: 535, 1953.
18. COHLAN, S. O.: Congenital anomalies in the rat produced by excessive intake of vitamin A during pregnancy. *Pediatrics* 13: 556, 1954.
19. MILLEN, J. W. and WOOLLAM, D. H. M.: Influence of cortisone on tetragenic effects of hypervitaminosis A. *Brit. M. J.* 2: 196, 1957.
20. WOOLLAM, D. H. M. and MILLEN, J. W.: Effect of cortisone on the incidence of cleft palate induced by experimental hypervitaminosis A. *Brit. M. J.* 2: 197, 1957.

Nutrition and a State Medical Society

THE ultimate goal of all research and clinical investigation in nutrition is the improvement of the health of the people. To this end several progressive state medical societies have established programs which vary in scope and objectives but which have as their fundamental philosophy the "practical application" of our newer knowledge of nutrition.

One of the leading examples of such a progressive viewpoint may be found in the report of the Commission on Nutrition of the Medical Society of Pennsylvania (*Pennsylvania M. J.* 60:1113,1957). The Commission's report may well be studied by appropriate commissions of other state medical societies for it represents an admirable example of what can and should be done.

In brief, the Commission has had two objectives: (1) The stimulation of interest in clinical nutrition at state and county levels; and (2) the dissemination of factual information on nutrition to both practicing physicians and the laity.

Among the numerous accomplishments of the Committee are the following:

A number of editorials on various aspects of applied nutrition appeared in the state medical journal.

Three exhibits were presented at the annual sessions of the state medical society. These dealt with obesity, electrolytes, and salt free diets.

A Coordinating Committee was established with representatives from city, state, and health medical societies, dental societies, state nurse organizations, etc. This Committee undertook a survey of the extensive nutritional training in hospitals. It was learned, not unexpectedly, that most hospitals offer little or no training in nutrition to interns and residents. The great majority of institutions, however, expressed a desire for a manual on standard therapeutic diets for both reference and teaching purposes. As a result, therefore, the Coordinating Committee prepared a manual of standard therapeutic diets which apparently has met with considerable enthusiasm state and nationwide. Copies of the manual have been distributed free of charge to senior medical students of the six medical schools in Pennsylvania.

As part of an education program, the State Nutrition Commission organized various symposia. These were held at State Medical Society meetings, and in cooperation with the National Vitamin Foundation, and with the Philadelphia County Medical Society.

The Commission has also urged the establishment of nutrition clinics throughout the state and supported a pioneering nutrition clinic now in operation at the Philadelphia General Hospital.

In cooperation with the Dietetic Association, a program will be prepared for the laity.

The Commission cooperated with the Pennsylvania Heart Association in the preparation of a manual on salt-free diets for free distribution.

The Commission also prepared a program of vitamin and other nutritional supplementation for patients under state public assistance. This doubtless will save the taxpayer a considerable amount of money.

The writer has had an opportunity to see a proposal for the establishment of a Division of Nutrition within the Department of Public Health of the City of Philadelphia. Because it is felt that this may be of interest to some of our readers, the following is a brief summary of this proposal.

The purposes of such a Division of Nutrition within a City Department of Public Health are (1) the promotion of better public health through research and special clinics; (2) the prevention of diseases arising from public ignorance of this field; and (3) direct participation in programs both in prenatal clinics, maternal nutrition studies, and school nutrition projects; and finally (4) rehabilitation of those who are in medical need from the public health standpoint, such as obesity, nutritional anemia, diabetes, etc.

The recommended organization would be located in a large city hospital. The staff would consist of a director, three physicians, two dietitians, a psychiatrist, a laboratory technician, two social workers, and a clerk. A number of laboratory studies would be performed including, in addition to routine determinations, analyses of vitamin C, urinary thiamine, urinary riboflavin, and electrolytes.

The Nutrition Division could plan courses for members of the Department of Public Health, such as public health nurses, dietitians employed by other state and city agencies, general physicians, and school physicians. Furthermore, this division could furnish consultants to other departments within and outside the Department of Health, would engage in the nutrition and education in medical schools and hospitals, help improve dietary practices in various state institutions, and advise various industrial hygiene divisions.

It is clear that a great deal may be offered by city- and state-organized medicine in improving the role of nutrition as a medical science and as an adjunct toward the health of the public.

A special commendation should be made to the Commission on Nutrition, State of Pennsylvania, under the chairmanship of Dr. Michael G. Wohl, whose members include Drs. T.

E. Machella, R. E. Olson, H. N. Seiple, J. N. Seitchik, P. L. Shallenberger, Paul C. Shoemaker, J. M. Strang, and C. W. Wirts, Jr.

It is hoped that through these means some of the progress in clinical nutrition can be brought to a more practical application to the health of the people.

—S. O. WAIFE, M.D

ANNOUNCEMENT

The Editorial Board of this Journal has long felt that the field of interest of clinical nutrition overlaps with the disciplines known as "metabolism" and "endocrinology." This historic but artificial separation seems meaningless now as current research has led to the abolition of boundaries between these three facets of man's adjustment to internal and external changes.

The Editorial Board, therefore, is pleased to announce the appointment of three Departmental Editors who will help in broadening the coverage of this Journal.

Metabolism: Kenneth R. Crispell, M.D., Associate Professor of Medicine, University of Virginia, Charlottesville.

Nutrition: D. Mark Hegsted, PH.D., Associate Professor of Nutrition, Harvard School of Public Health, Boston.

Endocrinology: Laurance W. Kinsell, M.D., Director, Institute for Metabolic Research, Highland Alameda County Hospital, Oakland, Cal.

Condolences

As we go to press, we learn with sorrow of the passing of Dr. Edward J. Stieglitz, a member of our Advisory Board. To the family of Dr. Stieglitz the publishers and editorial staff of this Journal extend their deepest sympathy.

Clinical Reports

Nutrition Surveys in the Near and Far East

REPORT OF THE INTERDEPARTMENTAL COMMITTEE ON NUTRITION FOR NATIONAL DEFENSE

FRANK B. BERRY, M.D. AND ARNOLD SCHAEFER, PH.D.

THE ENTHUSIASTIC and continued interest in nutrition improvement shown by the six countries in the Near and Far East where the Interdepartmental Committee on Nutrition for National Defense (ICNND) has conducted surveys during the past two years is evidence that this form of technical assistance is well accepted. During our visits to the Near East in the fall of 1956 and 1957, people repeatedly expressed thanks—and sometimes surprise—that the United States is interested in the welfare of men in other nations—in their health and their food. In most of the developing countries visited, the primary concern of the majority of the people is survival, which is dependent on food and shelter. These seem far more important to them than the possibility of war and destruction.

The work of the nutrition teams is carried out on a partnership basis, with scientists and technicians of the host country working with the United States members. The latter have reported that the nutrition studies are an effective form of scientific-military aid which often can be expanded and applied for improving the health of the civilians. The success of this type of mutual assistance is due in large part to the understanding, cooperation, and wholehearted support received from host country governments and military groups, U. S. Government agencies, and Military and Economic Assistance Missions.

Much credit is due our armed services, the U. S. Public Health Service, and the many universities and colleges that have released key personnel for the survey teams. The following educational institutions have participated: Vanderbilt, Cornell, Illinois, Maryland, Harvard, Rochester, Tulane, Temple, Agricultural and Mechanical College of Texas, Oregon State, Pennsylvania State, and Virginia Polytechnic Institute. The U.S. Army Medical Nutrition Laboratory, the National Institutes of Health, and the Office of the Surgeon General of the Army also have supplied personnel, while laboratory support has been obtained from the Naval Medical Research Unit No. 3 in Egypt and the 406th Medical General Laboratory in Japan.

We all are familiar with the role that food, nutrition, and health play both in a military operation and in daily life. The old saying that "An Army marches on its stomach" has not changed today. We may have atomic power to propel ships and generate electric power, but no such concentrated substitute for supplying man with energy has been developed. Calories for human propulsion and warmth are still derived from fats, proteins, and carbohydrates. Fuel and hardware are logistic problems, but food is a problem both of logistics and of health.

Sufficient data have been accumulated to

From the Office of the Assistant Secretary of Defense (Health and Medical), Department of Defense, Washington 25, D. C., and the National Institutes of Health, Bethesda, Md.

Published originally in the *United States Armed Forces Medical Journal* 9: 91 (Jan.) 1958, and reprinted with the permission of the authors and publishers.

state unequivocally that military equipment aid is a poor investment unless the personnel are physically capable of utilizing it with maximum effectiveness. This is not possible when the performance of some 50 per cent of the personnel is impaired by nutritional deficiencies, when training hours must be curtailed due to physical exhaustion, and when lack of a field-type ration reduces effective mobility regardless of the degree of mechanization of the armed forces.

During the past few years our Government has recognized the importance of food and nutrition as an integral part of the Mutual Defense Assistance Program of technical, military, and economic aid. At the close of World War II, the nutritionists of the United States and its allies were called upon to assist in Europe in diagnosing and recommending measures to alleviate problems of acute starvation associated with a secondary protein deficiency.

In Asia and Africa the nutritional problems are much more complex and their solution more difficult. To begin with, in sharp contrast to the large background of data available on Europeans, these is little factual information available on the nutritional conditions and requirements, food production, habits, customs, and taboos of many Asiatic populations. The countries included in the present program are keenly aware of the seriousness of their feeding and nutritional problems and have indicated a desire for assistance.

FORMATION AND FUNCTIONS OF THE ICNND

The Committee was organized as the result of a study of the Korean Army and of our efforts to assist the Chinese Nationalists on Taiwan in 1953–54. One of the findings was that much of the data in reference to food and nutrition which had been collected previously was not generally available to the groups having operating responsibilities in this area. No less than six different groups were independently making studies in Taiwan. A coordination of these studies was indicated to effect economy and to consolidate the recommendations.

In July 1954 an *ad hoc* coordinating committee on nutrition problems was organized at the National Institutes of Health, Bethesda, Md., under the sponsorship of the Department of Defense, with representatives of the departments and agencies with interest in and operating responsibilities for the Mutual Defense Assistance Program. The ICNND was formally established early in 1955 by a memorandum of agreement signed by the Secretaries and heads of departments of interested agencies (Departments of Defense—Army, Navy, Air Force—State; Health, Education and Welfare; and Agriculture; plus the International Cooperation Administration). It was later expanded to include the Atomic Energy Commission. Membership includes representatives from the above agencies.

The Committee has a Secretariat consisting of an Executive Director, a nutritionist, a clinician, and an agricultural economist, with offices at the National Institutes of Health. The late Dr. Harold R. Sandstead served as the first Executive Director until his untimely death in November, 1955. A panel of 20 consultants who are specialists in the fields of nutrition, medicine, biochemistry, food technology, and agriculture serves as an advisory body, and four subcommittees of consultants have been appointed to consider standard methods for nutrition surveys, nutritional requirements and working standards, nutrition research programs for foreign countries, and food and agriculture.

The purpose of the Committee is to deal with nutrition problems of technical, military, and economic importance in foreign countries in which the United States has a special interest. The Committee serves as a central clearing house on food and nutrition information, evaluates problems of food procurement and feeding, prepares reports and recommendations for the agencies concerned, reviews nutrition projects being conducted in areas where the United States is giving assistance, and coordinates, advises, and participates in field projects, when appropriate.

NUTRITION SURVEY PROGRAM

In April 1955 and in November 1956, representatives of the ICNND visited a number of

countries in Asia and Africa. Following these visits the Committee received requests from the governments of Iran, Pakistan, the Philippines, Turkey, Korea, and Libya for nutrition surveys of their armed forces. The first surveys were begun in January 1956 in Iran and Pakistan, and surveys now have been completed in all six countries. Funding was arranged through the Assistant Secretary of Defense for International Security Affairs as part of the Mutual Defense Assistance Program, the cost to the United States averaging only about $53,000 per country surveyed.

Objectives

The objectives of the nutrition surveys can best be summarized by three words: *assess*, *assist* and *learn*. The assessment phase involves an evaluation of the nutritional status of the population, and the capabilities and potential for improving the health of the people. In conjunction with the surveys, immediate assistance is given as follows: (1) by training host country personnel in nutrition evaluation technics, emphasizing clinical and biochemical phases and dietary intake and food production studies; (2) by furnishing essential laboratory equipment and supplies for establishing a permanent medical nutrition and food laboratory; and (3) by defining the major nutrition problems and developing practical recommendations so that the host country can best utilize the resources within the country.

The surveys afford an excellent opportunity for United States personnel to learn much from these countries regarding nutritional diseases, foods and food habits, and customs and practices. Also, the clinical, biochemical, and dietary data obtained contribute to a better understanding of nutritional diseases.

Implementation

Upon request for assistance from a country under the Mutual Defense Assistance Program, the ICNND organizes a nutrition team by appointing specialists in the fields of medicine, nutrition, food technology, agriculture, and sanitation. For most surveys the United States nutrition team members include a survey director, one or two clinicians, three biochemists, two food and dietary survey specialists (usually former U. S. Army nutrition officers), a food technologist and agricultural economist, and a sanitary engineer. The host country furnishes counterpart personnel, laboratory space, and other logistical support. About 70 to 90 days are required to make a survey.

Procedures

A *Manual for Nutrition Surveys* has been prepared to serve as a detailed guide for conducting surveys, and a revised edition, incorporating the experiences of the first four field studies, is in press at the U. S. Government Printing Office. The primary purposes of the *Manual* are: (1) to establish uniformity in methods, technics and procedures, so that a reliable comparison may be made of results of surveys within and among countries; (2) to serve as a reference to ensure maximum coverage of the major facts considered essential in appraising nutritional status to permit practical, effective recommendations; and (3) to define the responsibilities and duties of various team members.

The clinical team conducts a physical examination on a statistical sample, the number being determined by the population size. In general, about 2,000 persons are given a detailed examination, and an additional 3,000 to 5,000 are given abbreviated physical examinations that are limited to selected major signs of nutritional significance.

Samples of urine and blood are obtained from about 500 of the subjects receiving a complete physical. The biochemical analyses include determination of hemoglobin; hematocrit; plasma protein, vitamin C, carotene, and vitamin A; and urinary excretion of thiamine, riboflavin, and N^1-methylnicotinamide. The biochemical team is equipped to determine total serum cholesterol and serum albumin and globulin, and to conduct vitamin saturation tests if findings so dictate.

The food and dietary team determines daily food intake by inventory and food preparation survey technics, and collects composite food samples for chemical analysis. In addition, data are collected on food issues, menu plan-

ning, and food habits. The agricultural economist and food technologist surveys food production, preparation, processing, storage, and transportation.

HIGHLIGHTS OF SURVEY FINDINGS

General Observations

Although there are many similarities in the food and nutrition problems in the countries surveyed, each country must be considered separately and practical recommendations made for the solution of individual problems. Two factors affecting the nutritional status of developing countries are: lack of adequate transportation to move food from the area of origin to other parts of the country where these foods are in short supply; and lack of processed foods, resulting in considerable quantitative and qualitative fluctuation of food intake during different parts of the year. This lack of processed foods also curtails efforts to develop a field-type ration.

Cooking facilities in most of the kitchens consist primarily of large round-bottomed pots, either iron or tinned copper, often mounted over a dried clay firebox. Most kitchens prepare food for battalion-size units, and the meals are of the one-pot type, which is a stew or a soup. This is supplemented with bread in the wheat-eating countries, or a side dish of rice in the rice-eating areas. Typical kitchens are shown in Figures 1–3.

Other kitchen equipment usually is very meager, consisting primarily of stirring equipment, pails, knives, and a miscellaneous collection of containers. In most cases there are no mess halls, and each unit brings a large container to the kitchen where a measured amount of the food, depending upon troop strength, is issued. This is taken to the barracks, where the individual issue is made.

As one would expect, cooking facilities are less adequate with smaller units at outlying posts. In general, it is in these units that nutrition problems are most exaggerated. Most foods are procured from local contractors and in many cases fresh vegetables are purchased by individual units. Since refrigeration and preserved foods are extremely limited, virtually all perishables are issued on a 24-hour basis.

Improvement of health and sanitary conditions is dependent largely upon active participation and direction by the Command. In nearly all armed forces, this authority has been delegated. However, preventive medicine, which includes sanitation, housing, feeding and disease control, is of necessity a Command function.

Fig. 1. Typical "one-pot" system of cookery used in Iran and many neighboring countries.

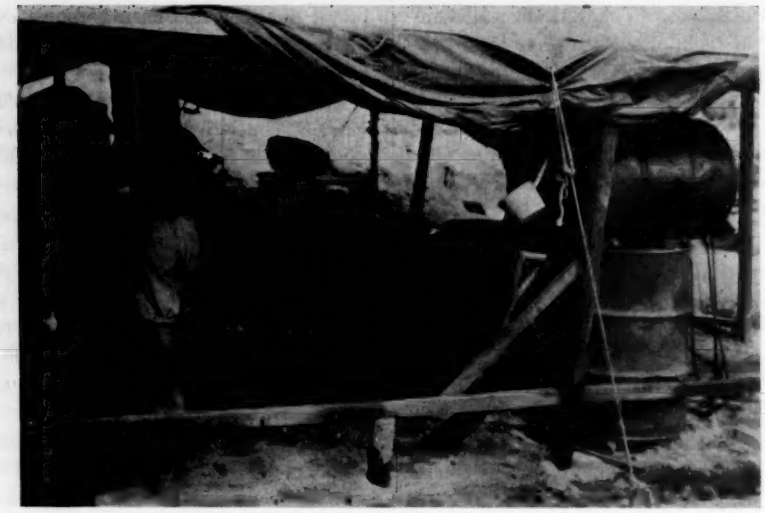


Fig. 2. Korean military field kitchen under canvas. The stove can be reconstructed quickly at a new location.

In most of the countries there is need for improvement in the basic ration allowance and in menu planning, with consideration given to the nutritional adequacy as well as to the cost of a ration.

In general, the principal nutritional deficiencies encountered in the countries surveyed were of riboflavin, thiamine, vitamin A, and vitamin C. Not every country has problems involving all of these vitamins, and in most cases, not all the areas within a country have such problems. Suboptimal intakes of these essential nutrients can, when sufficiently aggravated, result in serious physical disability of the troops. A man with scurvy, beriberi, or night blindness becomes a hospital patient and not an effective soldier.

General Recommendations

The teams recommend that a nutrition service be established, with representation from the medical, veterinary, and quartermaster

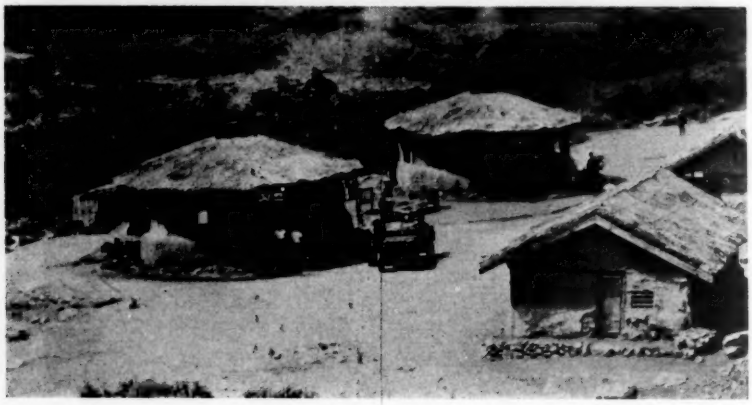


Fig. 3. More permanent type of Korean military field kitchen, with walls and thatched roof.

departments, and that this group establish close working relationships with civilian experts in the fields of nutrition, agriculture, food technology, transportation, and storage. Some of the principal duties of this service would be to assist Command in (1) formulating basic ration allowances and master menus for all the services, to ensure nutritional adequacy of all the essential nutrients; (2) developing and maintaining a list of food substitutes that could be used as a guide at the ration purchase and distribution points; (3) formulating and testing emergency field-type rations to be used in the field and, in some cases, at outlying posts; and (4) determining the nutritional status of the troops at periodic intervals.

In a few instances, food wastage presented a serious problem. Much of this resulted from overissue of certain foods that the troops did not like, or poor food preparation and, occasionally, poor quality of the food purchased. Where it did occur, the nutrition service gave invaluable help in correcting the situation.

In nearly all cases the nutrition problems encountered could be corrected by proper utilization of the resources within the country concerned.

Principal Nutrition Problems

A summary of the major clinical biochemical, and dietary findings is given in Table I. With a few exceptions, there is a good correlation between these three phases of the nutritional assessment.

Riboflavin: The most universal problem encountered and the most difficult one to correct by utilizing native foods was that involving riboflavin. The average dietary intake of riboflavin varied from 0.8 to 1.2 mg per man per day. In three countries (Korea, Iran, and

TABLE I

The Major Clinical, Biochemical, and Dietary Findings in Surveys of Armed Forces Personnel of Six Countries in the Near and Far East

	Korea	Iran	Pakistan	Philippines	Turkey	Libya
Clinical examinations (number)	1,514	1,730	2,019	4,234	8,519	1,749
Biochemical determinations (number)	303	396	440	454	329	144
Riboflavin nutriture						
With angular lesions (per cent)	13	15	0.6	0.8	23	5
With angular scars (per cent)	11	21	0.1	72	17	4
With urinary excretion <30 μg/6 hr (per cent)	16	21	3	7	5*	4
Average dietary intake (mg/day)	1	1.1	1.2	0.8	1.1	1.1
Thiamine nutriture						
With loss of ankle jerks (per cent)	0.1	1.3	0	3	1.3	1
With urinary excretion <25 μg/6 hr (per cent)	5	9	6	41	4	44
Average dietary intake (mg/day)	2.1	2.8	2.6	1.1	3.1	0.7
Vitamin C nutriture						
With scorbutic type gums (per cent)	0.3	1.6	0	0.3	5.8	0.1
With bleeding gums (per cent)	0.4	13	6	0.1	10	8
With serum vitamin C <0.2 mg/100 ml (per cent)	1	51	44	17	73	74
Average dietary intake (mg/day)	55	22	60	39	15	23
Vitamin A nutriture						
With follicular keratosis (per cent)	5	30	29	3	21	2
With serum vitamin A <20 μg/100 ml (per cent)	18	24	0.6	0.3	9	14
With serum carotene <40 μg/100 ml (per cent)	7	58	11	12	25	0
Average dietary intake (I.U./day)	1,218	3,766†	3,790	3,391	1,510	2,291

* An additional 9 per cent excreted <40 μg/6 hr, and 39 per cent <60 μg/6 hr.

† Range for areas 405 to 4,867.

Turkey) low dietary intake was accompanied by low urinary excretion of riboflavin and a high incidence of angular lesions.

In both the Philippines and Libya, it was surprising in view of the dietary intake data that the incidence of clinical indicator lesions of ariboflavinosis was very low. In reviewing the data, however, other factors must be considered, for in both countries the dietary intake of thiamine was about 30 to 50 per cent of that in the other four countries surveyed. If data from experimental animals can be applied to man, the observed urinary excretion of riboflavin may have been disproportionately high, inasmuch as it has been shown that chronic thiamine deficiency in rats tends to increase excretion of riboflavin. Regardless of the physiologic result or adaptation, the riboflavin intake must be considered deficient according to present-day knowledge.

Thiamine: Two of the countries surveyed (Korea and the Philippines) are primarily rice-eating populations. The observations shown in Table I indicate a marked difference in the thiamine nutriture of the population groups studied in these two countries.

In the Philippines highly polished rice, which greatly lowers the dietary thiamine intake, is used. Although there is provision for rice enrichment in the Philippines, only a small part of the rice purchased by the armed forces is enriched. In one area where enriched rice was used, the average dietary intake of thiamine was about 0.3 mg greater than in areas where unenriched rice was used, and the percentage of troops excreting low levels of thiamine was greatly reduced (from 29.5 per cent to 7.9 per cent). In Korea, the armed forces use only undermilled government rice, with a thiamine content of about 0.25 mg per 100 grams. However, most rice for civilian consumption is polished, with a thiamine content of only 0.18 mg per 100 grams.

Vitamin C: In general, the vitamin C status of the personnel examined varied greatly within the countries surveyed. In one area of Turkey, 40 per cent of the troops had blood serum vitamin C levels of less than 0.1 mg, in another area, only 2 per cent of the troops had correspondingly low levels. In four of the countries, a large percentage of those examined had low levels of serum vitamin C and dietary intakes were as low as 15 mg per man per day, although no cases of overt scurvy were noted. In some areas, however, the high incidence of scorbutic-type gums left little doubt that lack of vitamin C is a serious problem.

Vitamin A: There was a great variation in vitamin A intake within each country. In Iran the average intake was about 3,700 i. u., but with a range of from 400 to 5,000 i. u. per man per day. It is realized that dietary intake figures based on a relatively short period do not truly represent the vitamin A nutritional status of the individual, because vitamin A can be stored in the body for considerable periods of time. In most of these countries there are periods of fairly abundant supplies of vitamin A from leafy vegetables, followed by periods of short supply in the nonvegetable growing season.

ACCOMPLISHMENTS

It has been highly encouraging to note that following completion of the initial survey the countries have continued to carry out nutrition studies and have formulated and implemented practical recommendations that their own resources will enable them to carry out not just as an interim, but as a basic program. Evaluation of improvement in the nutrition and health of a population is difficult. It usually is not of the dramatic type where the value can be determined immediately. Final evaluation of a nutrition program can only be made by measuring progress over the ensuing years, noting the general improvement in the health and vitality of the people and in the over-all productivity.

Korea

One of the first opportunities to evaluate the benefit derived from a nutrition survey came in 1956 when President Syngman Rhee invited the ICNND to conduct a resurvey of the armed forces of the Republic of Korea. The initial survey had been conducted in 1953 under the auspices of the U.S. Army, Office of the Surgeon General, by a team headed by Dr. Harold R. Sandstead.

The dramatic improvement in the nutritional status of the Korean soldier as noted on resurvey is illustrated by some typical data given in Table II. The inductee of 1956 was virtually indistinguishable from the inductee of 1953, with the exception of a better vitamin C nutriture. However, there was a vast improvement in the nutritional status of the recruits after 16 weeks of training. It also was noted in 1956 that the longer the individual remained in service, the better his nutritional status became. These improvements were due, in considerable part, to the efforts of the Republic of Korea and the United States advisory personnel to carry out recommendations made following the 1953 survey.

As a result of the 1956 survey, it was decided that the refined wheat flour now provided by the United States will be enriched with 3.0 mg instead of 1.2 mg of riboflavin per pound. At the present rate of flour consumption in Korea, this will provide an increase of about 0.25 mg of riboflavin per man per day, at an additional cost of less than $500 per 100,000 men per year.

An example of Korean ingenuity in developing a practical mess gear adapted to their type of foods is shown in Figure 4.

TABLE II

Improvement in Nutritional Status of Korean Recruits in 1956 as Compared with 1953. All Figures Given Represent Percentages of Total Number of Armed Forces Personnel Examined

	1953		1956	
	Raw recruits	After 16 weeks' training	Raw recruits	After 16 weeks' training
Caloric status				
Below 90 per cent standard weight	27.0	46.0	29.0	26.0
90–110 per cent standard weight	72.0	53.0	71.0	67.0
Protein				
Leg edema	0.2	6.0	—	—
Serum protein <6 grams	—	11.0	—	—
Thiamine				
Calf tenderness	4.0	4.0	—	—
Vitamin C				
Scorbutic gums	21.0	46.0	—	0.6
Serum vitamin C <0.2 mg/100 ml	60.0	100.0	3.0	—
Vitamin A				
Follicular keratosis	3.0	13.0	5.0	4.0
Serum vitamin A <20 μg/100 ml	—	17.0	—	—

Iran and Pakistan

The accomplishments of the nutrition teams to Iran and Pakistan in January to April 1956 have borne results far beyond expectations. Both governments, as expressed through their departments of defense, are extremely grateful for the nutrition studies and the assistance of our Military Assistance Advisory Groups. Nutrition services have been established and are carrying out the recommendations of the teams to improve the nutritional status of their troops. Since the departure of the United States nutrition survey team, the Iranian team has conducted a thorough appraisal of the troops located in the Khash area, and additional surveys are under way. Iran also has rehabilitated a meat and vegetable canning factory and has plans under way for a second food cannery. This has made possible the development and production of a canned, stew-type field ration.

A quotation from the Health Director, United States Operations Mission, Pakistan, in the March 1956 issue of the *International Cooperation Administration Health Advisor*, is noteworthy:

> The entire program has benefited from the presence of an outstanding team of nutritionists furnished by

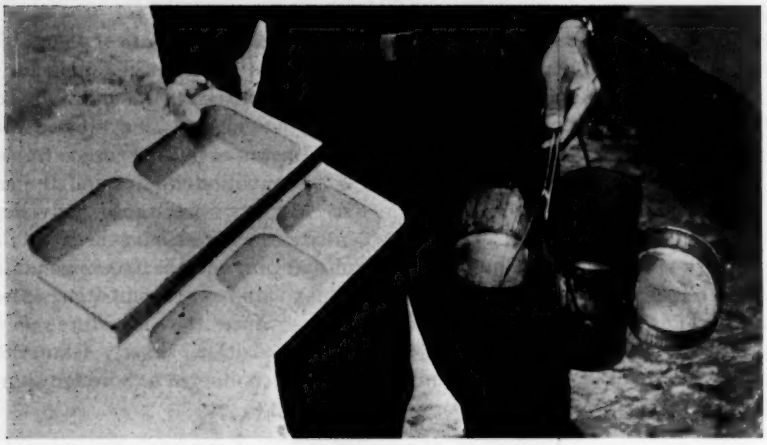


Fig. 4. New mess gear developed by the Koreans for their army.

the U. S. Interdepartmental Committee on Nutrition for National Defense. As a superbly organized action team doing large-scale survey work with the Pakistan Armed Forces in several parts of the country, they have been able to reawaken interest in nutrition problems at all levels. By devoting long hours to intensive laboratory work and arranging discussions with key civilian and military officials in government, they have discovered several well-qualified Pakistani biochemists and laboratory workers, some of whom eventually may form the nucleus for an effective national nutrition program.

International Nutrition Committee: As a result of contacts made by the United States nutrition survey teams in Iran and Pakistan, the Iranian government invited members of the Baghdad Pact to a general nutrition conference in Tehran in November 1956. The conference was attended by representatives of the Armed Forces of Iraq, Pakistan, Turkey, the United Kingdom, and the United States. The delegates passed a resolution recommending that an Internation Committee on Nutrition be organized on a permanent basis, with meetings to be held annually to discuss mutual nutrition problems. They requested the ICNND to serve as the secretariat for the first year of operation.

The United States Government, through the Departments of State and Defense, has authorized United States participation in the International Committee, and the governments of Iran, Pakistan, and Turkey have officially approved its formation. Plans recently were completed to hold the second meeting of the Committee in Turkey on April 7–9, 1958.

The hospitality of the Iranians, their sincere friendship, and the active participation of all delegates at the conference left a very favorable and lasting impression. This opportunity for discussing mutual nutrition problems, exchanging ideas, and learning of new technics set a precedent for later meetings of this type.

Turkey

Although the nutrition survey was completed only a few months ago, it already is evident that the Turkish team members were of a high professional caliber and exceedingly well trained to continue an active and progressive nutrition program in Turkey. Within a few weeks after the United States team members had departed, the Turkish nutrition team conducted an additional survey of another area. They forwarded to the Committee detailed findings covering the clinical examinations, laboratory findings, and dietary and ration studies. Their reports show that their team is highly competent, not only in assessing the nutritional status, but also in formulating practical recommendations for improvement.

The preliminary report of the joint nutrition survey was reviewed with the Turkish survey team in October 1957. They had arrived at conclusions and recommendations similar to those of the United States team, and were

actively investigating procedures and means for enriching wheat flour with riboflavin and increasing the fortification of margarine with vitamin A from the usual 400 to 500 i. u. per 100 grams to 8,000 i. u., with establishment of Government specifications and controls to ensure such a level.

The laboratory equipment and supplies furnished by the United States are being used not only in support of nutrition surveys but also in furthering basic research on the effect of nutrition in reference to diseases and parasitic infestations. A comprehensive study is under way of the vitamin content of some of the principal foods, especially as concerns vitamin A, vitamin C, and riboflavin. The possibility of using locally prepared rose cake as a source of vitamin C is under study. (This is a product made from rosebuds that was used extensively by the German submarine forces in World War II.)

Republic of China (Taiwan)

A report on "Rice Enrichment in Taiwan" was prepared by the ICNND and distributed to interested agencies, to implement the major recommendations for rice enrichment[1] that were made as a result of the nutrition survey of the armed forces of the Republic of China, conducted by the Office of the Surgeon General, U. S. Army, in the fall of 1954. This involved the establishment of two rice-enrichment, (Premix) plants in Taiwan. These plants were purchased recently and will be in operation in the summer of 1958.

In this method of enrichment, 1 part of rice Premix, which contains supplemental thiamine, riboflavin, niacin, and iron, is added to 199 parts of rice at the local mills, where feeders meter the Premix into the stream of polished rice. The additional cost of rice enriched by this method is less than one tenth of a cent per man per day.

As an interim method, the Committee suggested and assisted in planning a test in Taiwan of a new means of rice enrichment, using an enrichment wafer that is added to the rice during cooking. Since the method of cooking rice in Taiwan does not involve the use of excess water, the loss of soluble nutrients is negligible. Also, the recipe and equipment for preparing rice are uniform; about 20 kg of rice are prepared in one pot. Each wafer contains 40 mg of thiamine, 120 mg of riboflavin, 600 mg of niacin, and 400 mg of iron, which enriches the rice at a level similar to that to be attained by the Premix procedure. The cost of the wafer method is approximately $0.22 per man per year. A test completed in December, 1955 indicated that the wafer method of enriching rice was practical in Taiwan, and to avoid delay in improving the nutrition, this method was implemented in 1956.

Use of enrichment wafers has the disadvantage of requiring close supervision, stringent control, and cooperation by the mess personnel in the proper issue and utilization of wafers. Enrichment of rice is accomplished in thousands of mess halls, whereas with the Premix method control points number only about 100. Also the wafer method is particularly suited for use only under normal messing conditions where standard batch quantities of rice are prepared.

Good Will Engendered

Along with surveys that amalgamate scientific effort in numerous countries for the betterment of health and welfare, many intangible benefits accrue, although the mental and political responses as concerns good will, friendship, and better understanding are much more difficult than the physical benefits to evaluate. The creation of good will and understanding has not been one-sided. Returning United States nutrition survey team members are doing "double duty." They are serving as individual ambassadors for the countries visited. Friendships have been created of much longer duration than just the three-month tour. A part of what has been accomplished can be measured by the continued flow of correspondence, exchanging scientific information, requesting technical advice on specific problems, and reporting progress.

COMMENTS

Opportunity for Learning

Nutrition surveys in the developing countries

afford an opportunity for experience and training personnel not only of the host country but also of the United States team in a wide variety of nutritional disease patterns. Concurrent with present-day interest in the relationship of diet to heart disease, dental caries, cancer, and other conditions, the surveys present an excellent opportunity for further study of these problems. The program of the Committee anticipates conducting three nutrition surveys per year, with a continued follow-up in countries previously surveyed.

Need for Nutrition Research

The need for a more definitive, practical, laboratory method to assess riboflavin nutriture, especially in populations that are subsisting on suboptimal or borderline intake of thiamine, has been mentioned. The possible relation of low vitamin A intake to the incidence and severity of trachoma is of special interest in many areas of the world. A problem of considerable concern in the Near East is the high incidence of kidney stones. Is this of nutritional origin? Is the high incidence of parotid enlargement noted by Sandstead, Koehn, and Sessions[2] in Korea in 1953 of nutritional origin? This appears likely, since the incidence noted in the 1956 survey was markedly reduced.

In Libya a special nutrition condition was noted. The dietary intake of calories, riboflavin, thiamine, vitamin C, and vitamin A was at levels one would classify as suboptimal to deficient. There also was a high incidence of troops with low serum vitamin C and low levels of urinary excretion of thiamine and N^1-methylnicotinamide. However, the classic signs of active nutrition deficiency were present in only a relatively small fraction of the men examined. Based solely upon the physical findings, one would be obliged to interpret the data as indicating that no serious nutrition problems existed, but when the biochemical findings are considered together with the dietary intake studies it becomes apparent that nutrition problems do exist. These findings offer a challenge to nutrition researchers and emphasize the need for study in the field of suboptimal intakes of multiple nutrients and physiologic adaptation as related to health, work output, and resistance to disease.

Reports Prepared by the Committee

The following reports on individual nutrition surveys and suggestions for corrective action have been prepared by the Committee, and a limited number of copies are available on request from the Executive Director, Interdepartmental Committee on Nutrition for National Defense, National Institutes of Health, Bethesda 14, Md.

(1) Report on Mission to Near East, April 1955, by Dr. Frank B. Berry and Dr. Harold R. Sandstead.
(2) Trip to the Middle (Near) East, November 1956, by Dr. Frank B. Berry and Dr. A. E. Schaefer.
(3) Rice Enrichment in Taiwan, June 1955.
(4) Iran: Nutrition Survey of the Armed Forces, August 1956.
(5) Pakistan: Nutrition Survey of the Armed Forces, September 1956.
(6) Iran: Nutrition Survey of the Armed Forces. Supplement No. 1—The Khash Survey, December 1956.
(7) Korea: Nutrition Survey of the Armed Forces, April 1957.
(8) Philippines: Nutrition Survey of the Armed Forces, November 1957.
(9) Turkey: Nutrition Survey of the Armed Forces, December 1957.
(10) Libya: Nutrition Survey of the Armed Forces and Civilians, December 1957.

SUMMARY

The Interdepartmental Committee on Nutrition for National Defense was established early in 1955 by the Departments of Defense; State; Argiculture; and Health, Education, and Welfare, plus the International Cooperation Administration and (later) the Atomic Energy Commission. Its purpose was to deal with nutrition problems of technical, military, and economic importance in certain foreign countries. Nutrition surveys have been made in Iran, Pakistan, the Philippines, Turkey, Korea, and Libya, on request from the governments of these six countries. Some 12 American educational institutions have released key personnel for the survey teams. The deficiencies most commonly noted were of riboflavin, thiamine, and vitamins A and C; to some extent, of protein and total calories. Notable improvements in nutrition of armed forces personnel are being brought about by

host countries through actions based on recommendations made as a result of the surveys.

REFERENCES

1. Pollack, H. (editor): Symposium on nutrition: Studies on nutrition in the Far East. *Metabolism* 5: 203, 1956.
2. Sandstead, H. R., Koehn, C. J., and Sessions, S. M.: Enlargement of parotid gland in malnutrition. Am. J. Clin. Nutrition 3: 198, 1955.

The Geography of Alcoholism

"Alcoholism is not a problem shared equally by mankind. It is the curse of the richest communities and of the poorest who would use it as a solace. It is no respecter of race or culture, and it is sometimes difficult to see by what the habit is induced and fostered. It is a problem said to be comparatively slight in Oriental races. It is a problem of appalling size in the United States of America. In France the position yearly grows worse, and drastic government controls are contemplated. In Sweden liquor rationing was recently abandoned with no grave signs that the excesses of past years will recur. In Britain the law is relatively lax, and the problem is comparatively small and is said to be decreasing."
—*Med. J. Australia* 1: 977, 1956.

The Effects of a Late-Night Caloric Supplement Upon Body Weight and Food Intake in Man

JEFFREY H. FRYER, M.B., B.S. (LONDON)*

THE BASIC problem which underlies active obesity, when excess adipose tissue is being accumulated, is a failure of the organism to maintain equilibrium between energy intake, in the form of food, and total energy output. Both the taking in of energy and much of its expenditure are under voluntary control and the maintenance of a balance between these levels would seem at first sight to be a matter of chance. There is good evidence in the experimental animal, however, for the existence of one or more homeostatic mechanisms which, with varying degrees of efficiency, tend to regulate food intake in accordance with total energy expenditure and hence, in the adult animal, to maintain a more or less constant body weight under standard circumstances.

Gasnier and Mayer[1] studied the effects of this regulation in rabbits, showing that if they increased the animals' energy expenditure to maintain body heat, by shaving off their hair and exposing them to varying environmental temperatures, the food intake would be correspondingly increased. Without considering any particular mechanism which might be involved, these workers were able to determine some of the general characteristics of the regulation with regard to such parameters as precision, sensitivity, and reliability. Kennedy[2] has made similar studies on normal rats and upon rats made hyperphagic by the destruction of selected areas of the hypothalamus, demonstrating the immediate and delayed effects of hot and cold environments and also the response to an entirely natural circumstance increasing the energy output, lactation. Adolph,[3] working with rats, showed by the dilution of food with non-nutrient materials that, within limits, the animals ate for calories. Following periods of deprivation of food, body weight was slowly restored with the consumption of small excesses. Compensation for the caloric deficit was, however, largely achieved by a reduction in energy expenditure. This investigator also demonstrated the interaction of food and water balance underlining the over-all complexity of the regulatory mechanisms involved.

In experiments of a different nature, Janowitz and Grossman[4] and Share and co-workers[5] have studied the effects of feeding dogs with gastrostomies under various conditions, demonstrating that the introduction of more than 33 per cent of the normal caloric intake through the gastric fistula would depress oral intake to provide at least partial compensation for the intragastric calories. They concluded that total food intake was regulated in part by oral and gastric factors, including gastric distention, but that a systemic factor, reflecting caloric deficit or surplus, was also effective in accomplishing at least a coarse regulation of energy balance.

The only direct studies of a similar nature known to have been made in man were those of Hollander *et al.*[6] using a young male patient with esophageal obstruction and a surgical jejunostomy. Within limits their findings confirmed the relationship between metabolic needs and caloric intake, although the so-called oral component of appetite ultimately became so dominant that no valid conclusions could be

From the Graduate School of Nutrition, Cornell University, Ithaca, New York.

* Associate Professor of Clinical Nutrition, Cornell University, Graduate School of Nutrition, Ithaca, New York.

drawn which would be applicable to normal subjects. However, levels of energy expenditure may vary considerably in the human, e.g., in hot or cold climates or in daily occupations demanding more or less physical activity, and the general experience would indicate that caloric intake is frequently rather efficiently matched to requirements. The constancy of body weight in any specific population groups does not seem to have been documented: however, one has the impression that many individuals maintain a relatively constant weight, over long periods of time, without any conscious regulation of either food intake or energy expenditure. Conversely, however, any such regulatory mechanism in man must be potentially frail for, unlike most mammals of a lower order, obesity is commonly his problem.

This paper reports an experiment designed to test for a mechanism regulating food intake in man by a means not basically dissimilar from the dog experiments of Janowitz, Grossman, and co-workers (*loc. cit.*) It was carried out using college students who were actively following their daily routine during the project and this imposed certain limitations of time and method which were distinctly disadvantageous, e.g., food intake had to be estimated rather than measured objectively. However it is felt that the results demonstrate certain facts of potential importance with regard to the regulation of energy equilibrium in man.

PLAN OF PROCEDURE

Subjects

Subjects were 12 healthy male college students in the age range 18 to 25. These were selected from an initial group of 19 volunteers, all of whom were within normal weight limits as determined by the Standard Tables of the Metropolitan Life Insurance Company. Criteria of selection of the larger group also included a history of relative weight stability, a relatively constant level of voluntary energy output, e.g., in athletics, and a regular routine pattern of meal-taking. The weight fluctuations of the larger group were then observed by weighing under standard circumstances at weekly intervals for five weeks and a final group was selected at the end of this period on the basis of relative weight stability. The maximal weight fluctuation in any individual subject used in the study was 4.5 lb during the five-week observation period and the group mean maximal fluctuation was 2.4 lb.

Experimental Periods

The experimental period may be regarded as falling into three phases. The first phase was a period of observation of weight fluctuation on normal ad libitum diets lasting for six weeks (weeks 1 through 6). During the following three weeks (weeks 7 through 9) the subjects were given a "placebo" night supplement in the form of a noncaloric drink which was taken between 10 and 11 p.m. each night. Weeks 10 and 11 coincided with a university recess and subjects remained on an ad libitum regime without supplementation. The third and final phase started two weeks after the end of the second phase and lasted for eight weeks (weeks 12 through 19). During this period the subjects drank a 1,000-cal liquid supplement each night between 10 and 11 p.m. The constitution of the 1,000-cal and placebo supplements is given in Table I.

TABLE I

Constitution of Placebo and Caloric Supplements

a. Placebo supplement

Galactosol gum	2.5 g
Barium sulfate	10.0 g
Vanillin solution	5 ml
Sucaryl	5 ml
Water	to 500 ml

b. 1,000-calorie supplement

	Cal	CHO g	Protein g	Fat g
1 pint chocolate milk	449	54	17	19
75 g sucrose	289	75	—	—
23 g calcium caseinate	85	—	20	—
20 g corn oil	177	—	—	20
Total constituents	1000	129	37	39
Approximate % as calories	100	50	15	35

The basis of the 1,000-cal supplement was a pint of chocolate milk to which sucrose, casein, and corn oil were added to provide a ratio of 50 per cent carbohydrate, 15 per cent protein,

and 35 per cent fat calories. This was made up in bulk, passed through a homogenizer to prevent separation of the fat component, and stored in individual milk cartons containing exactly the volume required to provide 1,000 cal. The placebo mix was compounded of a gum base in water, with a small amount of barium sulfate added to lend "substance" and with artificial sweetening and flavor in the form of Sucaryl (Abbott) and vanillin. This was not intended to resemble the 1,000-cal mix as such, but to present to the uninitiated an artificial drink of totally undeterminable caloric content, hence, acting as a control to detect any effects due to the taking of a liquid supplement, *per se*, without regard to its caloric value. No information pertaining to either of the mixes was divulged to the experimental subjects at any time.

The subjects were told before the start of the experiment that they might experience a weight change in either direction, or that their weight might remain constant, but that in no circumstances were they to make *voluntary* efforts to reverse any trend but were to allow their food intake to be regulated only by the normal dictates of "appetite." Since no subjects had ever been obese, they were not expected to be unduly sensitive to the gain in weight which was actually anticipated.

Recorded Data

Weights were recorded at exact weekly intervals throughout all phases, being measured on the same scale to the nearest quarter pound without top clothes or shoes.

One-week dietary records were kept by the subjects during the second of the three weeks when the placebo supplement was being taken (week 8) and the first and seventh weeks of the supplemented period (weeks 12 and 18). Prior to these periods the subjects were given instruction and a demonstration to aid their estimation of quantities of foods commonly served. A record was also kept of periods of exertion, e.g., in sporting pursuits, over and above the routine daily round.

During weeks 8, 12, and 18 fasting blood sugar levels were determined on one day for all subjects and standard glucose tolerance tests were carried out on three subjects. Blood sugar was determined in venous and capillary blood by a modified Folin-Malmros method. Additional barium sulfate was added to the placebo or supplement mixes of all subjects on one evening of each of these three weeks and x-rays of the abdomen were taken at 7:30 to 8:00 a.m. the next morning to demonstrate the approximate extent of passage of the supplement through the intestinal tract.

RESULTS

Changes in Group Mean Weight

The observed changes in group mean weights throughout all phases are shown in Figure 1. The mean weight at the start of the caloric supplemented phase has been plotted as an arbitrary zero and other changes recorded as differences from this level. Additional curves are plotted showing the reactions to supplementation of two subgroups which are further discussed later in this paper.

The group exhibited a relatively stable weight during the initial phase of observation, lasting for six weeks, a mean gain of slightly more than 1 lb occurring in the last two weeks of this period. The subjects were, of course, selected with this stability as a main criterion. During weeks 7 through 9, when the placebo supplement was being taken, the mean weight increased by less than half a pound, so that, as would be expected, the ingestion of a "blank" mix amounting essentially to a pint of water did not affect the normal maintenance of weight stability previously demonstrated.

The introduction of a nightly supplement equivalent to 1,000 cal produced a substantial change in the weight curve as seen during the final eight weeks of the project. The group mean weight gain during the first week of this phase was 2.1 lb and in the second week 1.17 lb. A mean weekly gain of about 0.75 lb was then maintained over the three subsequent weeks. During the sixth week of this phase (week 17 of the whole experiment) a slight mean weight loss was recorded which coincided with, and is no doubt explained by, a long social week-end at the University, during which meals were eaten at irregular intervals and were not infrequently missed altogether. However during

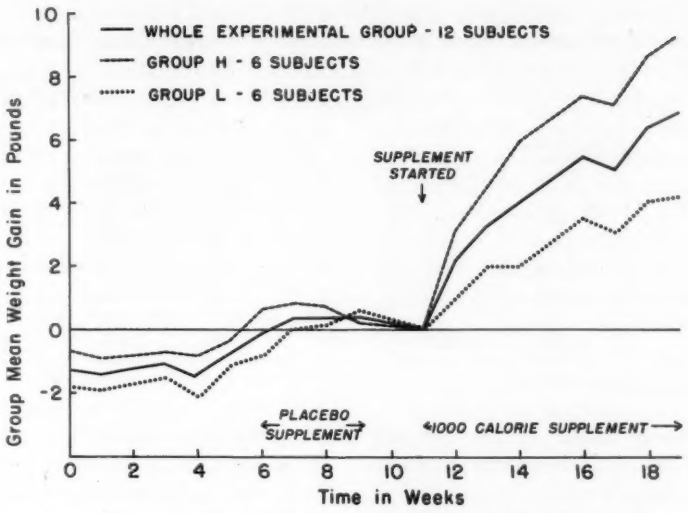


Fig. 1. Changes in group mean weight during periods of control observation, placebo, and caloric supplementation.

the subsequent week the mean weight gain was 1.23 lb, thereby "compensating" for the dip at week 17, and, with the final mean gain of 0.68 lb in the last week of the experiment, producing a rather smooth curve representing a gradual reduction in the weekly weight increment with the passage of time. The group mean weight gain during the supplemented period as a whole was 0.86 lb a week, compared with 0.11 lb a week during the 11 weeks when weights were observed but no supplement was given. This difference is statistically highly significant.

Analysis of variance showed that weight variation among subjects was the largest single component in both unsupplemented and supplemented phases and this was of approximately the same proportions in both phases. This, of course, reflects the fact that the subjects were of varying body weights irrespective of the experiment. During the supplemented phase the "within subjects" variation showed highly significant components attributable to linear regression and quadratic regression which are apparent in the mean curve shown in Figure 1.

Dietary Intakes During Weeks 8, 12, and 18

The mean caloric intakes of the experimental group during the second week of the phase of placebo supplementation (week 8) and the first and seventh weeks of 1,000-cal supplementation (weeks 12 and 18) were calculated from one week dietary records, using standard tables of food composition. Results are shown in Table II.

This shows a fall in the daily caloric intake (excepting the supplement) of 509 cal during the first week of supplementation and 467 cal during the seventh week. When analyzed by individual meals, however, there is a slightly different pattern between these two weeks in that the largest reduction in calories seems to have occurred at supper during the first week of supplementation, while breakfast was more affected during the seventh week. The most important comparison, however, is that between the net reduction in calories noted, approximately 500 daily, and the supplement given, which was 1,000 cal. From these figures it seems that whatever regulatory mechanism may be involved in maintaining a constancy of caloric intake (in circumstances of relatively constant energy output), this was approximately 50 per cent efficient for the group as a whole under the conditions of this experiment and its efficiency did not change in the two weeks observed over a seven-week interval.

Table III shows an analysis of the changes in

TABLE II

Changes in Food Intake at Regular Meals Ascribable to Late Night Caloric Supplementation

Meal	Week 8 placebo supplement mean intake *cal/day*	Week 12 1,000-cal supplement first week			Week 18 1,000-cal supplement seventh week		
		Mean intake *cal/day**	Changes from week 8		Mean intake *cal/day**	Changes from week 8	
			Cal	%		*cal*	%
Breakfast	676	609	−67	−10	474	−202	−30
Lunch	1,013	913	−100	−10	907	−106	−10
Supper	1,169	912	−257	−23	1,018	−151	−13
Late night snacks	199	110	−89	−45	104	−95	−48
Other snacks	73	77	+4	+5	160	+87	+119
Total	3,130	2,621	−509	−16	2,663	−467	−15

* Excluding supplement.

the weekly caloric intake of the subjects, grouped by the extent of the change, and data on the weight changes which occurred simultaneously. One subject apparently ate more during both the first and seventh weeks of actual supplementation than when receiving the placebo supplement and he gained an average of 4.25 lb each week. Three subjects did not essentially change their intake at regular meals during the first week of supplementation and the simultaneous mean weight gain was 3.83 lb/week. The comparable mean weight gain for the four subjects who decreased their intake by 1,000–3,000 cal is substantially less than would be expected, this being the only figure which does not fall in the sequence of steadily decreasing weight increments with decreasing caloric intake, if the figures for both weeks be combined. With this exception, the more efficiently the subjects "compensated" for the late-night supplement, by reducing their calories at other meals, the less weight they gained as a result of the supplementation. However, pooling the total experience of 24 subjects/weeks, almost 80 per cent of the subjects showed a decrease of more than 1,000 cal a week and 63 per cent of the subjects had a decrease of more than 3,000 cal a week. During only three subject weeks was there complete compensation, i.e., a reduction of intake at regular meals by the amount of the supplement, 7,000 cal/week.

Other Observations

Fasting blood sugar levels were determined on separate samples of capillary and venous blood from all subjects on one occasion during each of weeks 8, 12, and 18. Mean capillary levels were 92.0, 93.4, and 93.8 mg/100 ml, respectively, and comparable venous levels 90.25 90.0 ml, and 89.8 mg/100 ml. Differences are within one standard deviation in all cases and it is clear that no significant change in these levels, or in the capillary-venous (or arterio-

TABLE III

Correlation Between Change in Weekly Caloric Intake and Weight Gain (Weeks 12 and 18)

Changes in weekly caloric intake (excepting supplement)	Number of subjects			Mean average increase *lb/week*		
	Week 12	Week 18	% of total	Week 12	Week 18	Weeks 12 and 18
Decrease more than 7,000 cal	2	1	13	0.00	1.25	0.42
Decrease 5,000–7,000 cal	2	2	17	1.00	0.38	0.69
Decrease 3,000–5,000 cal	4	4	33	2.62	0.69	1.66
Decrease 1,000–3,000 cal	0	4	17	—	0.75	0.75
Unchanged within 1,000 cal	3	0	13	3.83	—	3.83
Increase more than 1,000 cal	1	1	8	2.00	6.50	4.25
Total	12	12	101	2.17	1.19	1.67

venous) differences can be attributed to the process of caloric supplementation.

Glucose tolerance tests on three subjects, carried out at similar times to the fasting blood sugar determinations, showed lower capillary and venous reponses to a standard glucose load during the supplemented period. Such a result could be attributed to an increase in over-all carbohydrate intake. However the Δ-glucose levels were essentially unchanged in the first one and a half hours and subsequently were maintained at a higher level during the unsupplemented regime.

X-rays of the abdomen taken some nine hours after the ingestion of placebo and 1,000-cal supplements, with added barium sulfate, showed that during either regime, none of the mix remained in the stomach after this period of time, and the majority of the barium itself was in the lower small bowel and the large bowel.

Recorded activity levels showed no changes likely to be significant in any of the subjects throughout the project.

DISCUSSION

The simple observation of weight trends under the circumstances of the experiment here described permits two basic conclusions. First, that the addition to the regimen of the experimental group of a 1,000-cal drink taken between 10 and 11 p.m. each night, produced an increase in the group mean body weight, as it would be expected to do. This is of statistically significant dimensions and may be ascribed to the additional calories *per se*, since it did not occur with a noncaloric placebo supplement. Second, as can be seen in Figure 1, the rate of weight gain produced in this way was not constant but, rather, could be represented by a curve tending to return toward the horizontal, indicating a progressive but irregular reduction in the weekly weight increment produced by the diet supplementation. Since the conditions of the experiment allow the assumption that the energy expenditure of the subjects remained more or less constant throughout, the changing weight increment may be ascribed to one or both of two main factors, a changing net caloric intake or changes in the nature and caloric value of the "excess" tissue laid down. Examination of the data on dietary intake enables some conclusions to be drawn about these two possible mechanisms.

The daily dietary intake during weeks 12 and 18, the first and seventh weeks of supplementation, was reduced by 509 and 467 cal, respectively, when compared to week 8, when the placebo or noncaloric supplement was being taken (See Table II). Since the caloric value of the supplement was 1,000 cal a day, the net increment attained by adding this fourth "meal" to the regime was between 491 and 533 cal a day. The difference between these figures cannot be taken as significant, representing less than 2 per cent of the daily intake. In contrast to the relatively constant level of dietary intake noted, however, the comparable mean weight increments for weeks 12 and 18 were 2.17 and 1.19 lb, respectively, these figures being corrected for the slight change in group weight during week 8, when the control observations were made. From these figures the caloric value of the tissue gained during the first week of supplementation is 3.4 cal/g and during the seventh week 6.9 cal/g. It is thus clear that more fluid was being retained during the first week than during the seventh. This finding is in general agreement with the work of Dole and others[7] who reported the caloric value of tissue gained and lost by obese women in alternating four-day cycles to be about 2.5 cal/g. This they contrasted with the 8 cal/g reported by Strang and Evans[8] as the caloric value of tissue lost by obese subjects over periods of several weeks. The findings of the present project must be regarded as only approximate, since food intakes were estimated rather than measured, however the trend from lower to higher levels of caloric "density" is in reasonable conformity with these previous observations.

It is unfortunate that week 18, the seventh week of supplementation, was not entirely representative of the group trends during the latter part of the supplemented phase as a whole, since there was a "rebound" from the previous week when the group weight decreased, probably due to the social activities of the subjects. During week 18, the weight gain

was more than double the mean gain for weeks 16 through 19, the last four weeks of supplementation. It is conceivable therefore that the dietary intake recorded for this week was higher than it would otherwise have been. This being the case, compensation for the additional calories of the night supplement, by reduction of the intake at other meals, would be better at the seventh week of supplementation than at the first and this would, in part, explain the diminishing weight increment occurring as the supplemented phase progressed. As things stand, however, there is no evidence that such compensation changed in degree, having a mean efficiency for the group as a whole of approximately 50 per cent in both the first and the seventh weeks of supplementation. The changing weight increment noted can thus be ascribed only to the steadily changing caloric value of accumulating "excess" tissue.

Data have already been presented for groups of individuals showing the association between the decrease in calories ingested, other than the supplement, and weight gained (Table III). It is of interest also to look at the individual results shown in Table IV. This shows the wide variation in the effectiveness with which individuals compensated for the additional calories in the night supplement, an index of which has been derived by the ratio of change in weekly caloric intake (excepting the supplement) to the 7,000 cal in the supplement, expressed as percentage compensation. This figure varies from −37 to +149 per cent for individual subject/weeks, the mean being +49.1 per cent. A negative compensation implies that the subject ate more during the supplemented weeks observed than the unsupplemented week which was used as a control. This is seen, in significant degree, in only subject 1, whose relative weight gain during the periods in question was almost equal to the net weight gained throughout the whole eight weeks of supplementation. It seems likely therefore that, for this subject, these two weeks were quite atypical.

For the group as a whole significant correlation between percentage compensation during the first and seventh weeks of supplementation cannot be demonstrated (r = 0.31). However, as implied by the summarized results of Table III, a correlation is seen between the net change in caloric intake in these weeks, and the simultaneous weight gain or loss (r = 0.54**). Furthermore, if the data for subject 1 be excluded because of the evidence indicating a

TABLE IV

Changes in Food Intake, Calculated Compensation for Supplemental Calories, and Net Weight Gain in Individual Subjects

Subject No.	Changes in intake (excepting supplement) *cal/week* (1)		Net change in intake (including supplement) *cal/week* (2)		% compensation for supplement* (3)			Total weight gained in lb (8 supplemented weeks) (4)
	Week 12	Week 18	Week 12	Week 18	Week 12	Week 18	Mean	
1	+2,200	+2,600	+9,200	+9,600	−32	−37	−34	6.75
2	−6,100	−4,900	+900	+2,100	87	70	79	4.00
3	−3,300	−1,300	+3,700	+5,700	47	19	33	10.25
4	−5,100	−4,200	+1,900	+2,800	73	60	66	4.25
5	−10,400	−3,800	−3,400	+3,200	149	54	101	5 5
6	−4,400	−5,800	+2,600	+1,200	63	83	73	8.5
7	+100	−6,400	+7,100	+600	−1	91	46	10.5
8	−3,300	−1,600	+3,700	+5,400	47	23	35	4.75
9	−4,900	−7,300	+2,100	−300	70	104	87	4.75
10	+900	−1,800	+7,900	+5,200	−13	26	6	10.25
11	−300	−3,500	+6,700	+3,500	4	50	27	9.75
12	−8,500	−1,300	−1,500	+5,700	121	19	70	3.00
Mean	−3,591	−3,275	+3,409	+3,725	51.2	46.8	49.1	6.85

* % compensation for supplement = $\frac{\text{reduction in intake (cal/week)(col. 1)} \times 100.}{7{,}000}$

highly atypical "negative" compensation during the weeks in question, a statistically significant percentage negative correlation exists between the mean compensation during weeks 12 and 18 and the total weight gained throughout the whole eight weeks of supplementation ($r = -0.65^*$). Such findings conform, of course, to anticipated results, but they are presented to support acceptance of the dietary intake data for the group as a whole, in spite of the necessarily crude method used in its collection. They suggest also that the dietary data in the weeks sampled were generally typical of the project as a whole, with the probable exception of subject 1.

On an individual basis the apparent degree of compensation attained was not entirely in accordance with the weight trend as evidenced, for example, by subject 5, whose compensation was recorded as 101 per cent, but who gained 5.5 lb in weight. This is explicable in three ways, any one or more of which may be responsible for such seeming discrepancies. The data on dietary intake may be in error, or may not be representative during the weeks sampled, as is suspected in the case of subject 1. In addition, however, the caloric value of accrued tissue has been shown to differ considerably for the group between weeks 12 and 18 and there is no reason why it should not have differed between individuals. Thus it is theoretically possible that subject 5 gained 5.5 lb of water only and his caloric compensation was indeed perfect, although one suspects this is not the whole explanation.

Because of these uncertainties direct comparison between individuals is not justified. However, it is of interest to compare the group of six subjects whose individual weight gain was less than 6 lb during the whole project (group L—low) with the six subjects who gained more than 6 lb each (group H—high), mean total gains for these two groups being 4.2 and 9.3 lb, respectively (see Fig. 1). The patterns of weight change during the unsupplemented phases were essentially similar for these groups although the group L subjects showed a greater increase in observed weight during this control period. However, during the first four weeks of caloric supplementation the relative weight increments were substantially different and the trends noted, both for individuals and subgroups L and H, had a high predictive value for their ultimate degree of weight gain. Five of the six individuals in each subgroup remained consistently in their own half of the group as a whole, when rated in order of total weight gained. In the last four weeks of supplementation, however, the mean weight gained by the subgroups L and H was almost identical and it is apparent that the real differences in the reaction of subjects to supplementation were maximal at the start of the supplemented phase and subsequently gradually diminished. During the first week of supplementation (week 12) group L subjects gained an average of 0.96 lb each and with 91 per cent compensation for the additional calories fed, the caloric value of accrued tissue was 1.5 cal/g. In contrast, group H subjects gained an average of 3.38 lb and showed only 11 per cent compensation, accruing tissue of caloric value 4.05 cal/g. The implication of these figures is that those who compensated more effectively also retained more fluid in ratio to true adipose tissue. Since both the degree of compensation and the caloric value of accrued tissue are dependent upon the measurement of net food intake, this relationship may be spurious. However, the possibility is of interest since several workers have suggested a link between variations in cellular hydration and a satiety mechanism.[9,10] During the seventh week of supplementation all differences between the subgroups had narrowed, the compensation being 55 and 39 per cent for the L and H groups, respectively, and the comparable caloric value of tissue gained was 8.4 g and 6.2 cal/g. No certain significance may be attributed to such differences in view of the methods used in collecting dietary data.

The ultimate potential importance of these observations is merely to underline the differences noted in the individual reaction to supplementation, particularly in regard to the effectiveness with which the "extra" calories were compensated by reduction in intake at regular meals. The supplement used in this experiment was approximately the equivalent of three ice-cream sodas, as available in every

drugstore, and seemingly subject 12 could indulge himself to this extent every night for eight weeks without running a great risk of initiating a trend toward obesity. He demonstrated, in fact, a high degree of obesity-resistance resulting from an efficient homeostatic mechanism tending to maintain energy balance. Subjects 3, 7, and 10 were clearly less obesity-resistant, but they too were selected as being usually of normal and relatively constant weight and so presumably their over-all homeostasis was good by common standards. Their performance must be contrasted with that of the obesity-prone individuals who seem all too common in Western populations and to whom any additional between-meals calories seem inevitably to add to their burden. Such people have to regard even one ice-cream soda as a threat to their efforts to reduce or keep weight down and the maintenance of energy equilibrium seems to be more a matter either of chance or of coarse voluntary control than of "automatic" regulation by a homeostatic mechanism.

Many theories have been proposed to explain variations in hunger-satiety in the human, including mechanisms dependent upon the mechanical effects of food in the gastrointestinal tract,[4,5,11] changes in levels of available blood sugar (glucostasis)[12] the effects of specific dynamic action (thermostasis),[13] and changes in cellular hydration.[9,10] Notwithstanding other objections, major or minor, which have been raised to these theories, it is of interest to examine their possible application to the results of the present experiment.

The data show that compensation for the additional calories given to the subjects between 10 and 11 p.m., occurred at all three meals the following day (Table II). Any later effects, beyond the 24-hour period, would not be detected since the supplement itself was given daily. The supplement was given at a late night hour so that at least eight hours would elapse before the next normal meal was taken and measurement of pre-breakfast blood sugar levels during the supplemented and unsupplemented phases showed that these were not affected by the additional calories taken the preceding night. The blood sugar response to ingested carbohydrates, as tested by a standard glucose tolerance test, was somewhat lower during the supplemented phase and Δ-glucose levels were slightly less well maintained than during the unsupplemented phase. In all respects these findings make it impossible to explain the compensation on the basis of a glucostatic mechanism, even for the meal immediately following the supplement. More delayed effects are even less explicable, underlining one of the fundamental defects of this theory as originally postulated, namely, that the blood-sugar level, in any form, reflects only recent intake of carbohydrate and is no index of total energy intake. This must essentially be one of the variables acting upon, and potentially influenced by, any hypothetical homeostatic mechanism thought to play a role in maintaining energy equilibrium.[14,15]

The x-ray studies in this experiment demonstrated, as would be expected, that there could be no possible mechanical influence of the liquid supplement upon food intake at normal meals the next day. The stomachs of the subjects were empty and the barium sulfate used to mark the progress of the mix was generally low in the gastrointestinal tract. Absorption of the nutrients in the supplement would be expected to be complete within the approximately nine hours which followed the ingestion of the supplement and preceded the next normal meal. It is more difficult to assess the possible effects upon food intake of specific-dynamic action or changes in cellular hydration ascribable to the supplement. These metabolic sequelae normally follow within a few hours of the time of ingestion of nutrients and the cycle is presumably largely complete, through absorptive and post-absorptive phases, within the normal between-meal period of perhaps four or five hours. However, the effects of the supplement might be cumulative and thus explain its depressant effect upon food intake 20 hours after ingestion. Such an explanation would require investigation. Meanwhile it should be noted that convincing objections have been raised to the "thermostatic" theory on other grounds.[2,16]

The apparent relationship between fluid retention and effectiveness of caloric compensa-

tion seen in group L and group H subjects in the present experiment may not be valid for reasons which have been given. However, the theory that the dehydration of specific cells, not as yet identified, is in some way associated with satiety[10] is seemingly contrary to the trend noted in the current experiment, at least as this applied to over-all fluid balance. In general, it should be noted also that more fluid is lost from the body during the earliest stages of weight reduction than later on, if the process is maintained, and it is during the earliest stages that the desire to eat is maximal. To this extent generalized relative dehydration is accompanied by hunger rather than anorexia. Specific tissues might of course be affected in a different manner.

It is finally pertinent to record the few observations on the weights of subjects which could be obtained after the supplemented phase was over, when the project as a whole had been discontinued. One subject had continued to gain weight one week after the cessation of supplementation. However, four others showed weight losses which were substantial in relationship to previously noted fluctuations. The total experience indicated a mean weight loss of 2 lb during each of the two weeks following the end of the supplemented phase. One would postulate that had it been possible to observe the whole group for some weeks at this time, the mean weight curve would have been an inversion of that recorded for the supplemented phase, with a rapid initial fall in weight due to the loss of accrued tissue of relatively low caloric value and only partial recompensation for the calories suddenly removed from the regimen. This would be followed by a gradual re-establishment of energy equilibrium at the originally stable weight level.

SUMMARY AND CONCLUSIONS

An experiment is reported in which 12 male college students, of normal and relatively constant weight, were given a 1,000-cal liquid supplement between 10 and 11 p.m. each night for eight weeks. The body weights of the subjects and their food intakes during two selected weeks of this period are contrasted with suitable control observations made when no caloric supplement was being given.

The group mean weight showed a statistically significant increase as a result of the caloric supplementation, the pattern of weight increase showing a decreasing weekly weight increment with the passage of time. The possible causes for this are discussed and evidence is presented that the caloric value of the "excess" tissue laid down was increasing, i.e., weight gain represented varying degrees of fluid retention, the proportion of true adipose tissue increasing as the period of supplementation was maintained. This is in accordance with the findings of other workers.

The group mean caloric intakes at regular meals decreased by approximately 500 cal a day during both the first and seventh weeks of supplementation. The "compensation" achieved for the 1,000-cal supplement was therefore approximately 50 per cent for the group as a whole and no evidence was adduced that this changed in degree with the passage of time. Variation in percentage compensation between individuals was however considerable, and a significant negative correlation was demonstrated between the effectiveness of compensation and net weight gain over the supplemented period.

The significance of these findings is discussed in terms of the variation in obesity resistance and obesity proneness which results from the greater or lesser efficiency of the homeostatic control of energy equilibrium.

Observations of blood sugar levels and rates of gastric emptying made during the control and supplemented phases of the project showed that the decreased intake of food at normal meals attendant upon late-night caloric supplementation could not readily be attributed to a glucostatic mechanism of appetite control or to a mechanism dependent upon the mechanical effects of nutrients in the gastrointestinal tract

ACKNOWLEDGMENTS

The author wishes to record his thanks to the experimental subjects who cooperated in the project here reported, to Mrs. Grace Fiala who carried out the laboratory work, Miss Dolores Michaud for the dietary computations, and to Dr. R. G. D. Steele who helped with the statistical evaluation of data.

REFERENCES

1. GASNIER, A. and MAYER, A.: Recherches sur la regulation de la nutrition. II. Les méchanismes regulateurs de la nutrition chez le lapin domestique. *Ann. Physiol. Physicochim. Biol.* 15: 157, 1939.
2. KENNEDY, G. C.: The role of depot fat in the hypothalamic control of food intake in the rat. *Proc. Roy. Soc.* (*B*) 140: 578, 1952–53.
3. ADOLPH, E. F.: Urges to eat and drink in rats. *Am. J. Physiol.* 151: 110, 1947.
4. JANOWITZ, H. D. and GROSSMAN, M. I.: Some factors affecting the food intake of normal dogs and dogs with esophagostomy and gastric fistula. *Am. J. Physiol.* 159: 143, 1949.
5. SHARE, I., MARTYNIUK, E., and GROSSMAN, M. I.: Effect of prolonged intragastric feeding on oral food intake in dogs. *Am. J. Physiol.* 169: 229, 1952.
6. HOLLANDER, F., SOBER, H. A., and BANDES, J.: A study of hunger and appetite in a young man with esophageal obstruction and jejunostomy. *Ann. New York Acad. Sc.* 63: 107, 1955–56.
7. DOLE, V. P., SCHWARTZ, I. L., THORN, N. A., and SILVER, L.: The caloric value of labile body tissue in obese subjects. *J. Clin. Investigation* 34: 590, 1955.
8. STRANG, J. M. and EVANS, F. A.: The energy exchange in obesity. *J. Clin. Investigation* 6: 277, 1928.
9. HOELZEL, F.: Appetite and obesity. *Am. J. Digest. Dis.* 12: 156, 1945.
10. LEPKOVSKY, S., LYMAN, R., FLEMING, D., NAGUMO, M., and DIMICK, M.: Gastrointestinal regulation of water and its effect on food intake and rate of digestion. *Am. J. Physiol.* 188: 327, 1957.
11. PAINTAL, A. S.: A study of gastric stretch receptors. Their role in the peripheral mechanism of satiation, of hunger and thirst. *J. Physiol.* 126: 255, 1954.
12. MAYER, J. and BATES, M. W.: Blood glucose and food intake in normal and hypophysectomized, alloxan-treated rats. *Am. J. Physiol.* 168: 812, 1952.
13. STROMINGER, J. L. and BROBECK, J. R.: A mechanism of regulation of food intake. *Yale J. Biol. and Med.* 25: 383, 1953.
14. FRYER, J. H.: Blood glucose response to the energy-yielding nutrients. *Fed. Proc.* 15: 552, 1956.
15. FRYER, J. H., MOORE, N. S., WILLIAMS, H. H., and YOUNG, C. M.: A study of the inter-relationship of the energy-yielding nutrients, blood glucose levels and subjective appetite in man. *J. Lab. & Clin. Med.* 45: 684, 1955.
16. MAYER, J.: Regulation of energy intake and the body weight. *Ann. New York Acad. Sc.* 63: 15, 1955.

The Acute Reduction in Plasma Amino Acids by Carbohydrate Infusion in Diabetes and Liver Disease

MILTON E. RUBINI, M.D.* AND DAVID SELIGSON, M.D.†

EXERCISE, insulin, and glucose feeding share a propensity to acutely reduce the plasma amino acid nitrogen in fasting normal animals and man.[5,6,12,16,18,26] This has been construed as evidence of an intimate interrelation of increased utilization of carbohydrate with a net gain in the metabolism of protein.[20,25] The hypothesis that this acute decrease in the plasma amino acid pool reflects either an increase in protein synthesis, or a decrease in protein breakdown has not been delineated.[1,4,20,21,25] By the use of specific microbiologic technics, it has been shown that individual amino acids are depressed unequally, and Munro and Thomson[21] have suggested that inasmuch as the proportions by which individual amino acids were reduced were similar to the relative proportions of these amino acids required for protein synthesis, carbohydrate feeding effects a change in the metabolism of the *entire* protein molecule.

The present study confirms the acute reduction in plasma amino acids of normal men by measures which increase carbohydrate utilization. By substituting fructose as the infused carbohydrate in normal individuals, and extending the observations to include a group of probable insulin-deficient diabetics, evidence is offered that this phenomenon is independent, to a large degree, of insulin action. In addition, the behavior of plasma amino acids following carbohydrate infusion to a group of patients with liver disease and the response in dogs with fatty metamorphosis of the liver induced by choline deficiency are presented.

From the Department of Metabolism, Division of Medicine, Walter Reed Army Institute of Research, Walter Reed Army Medical Center, Washington, D. C.

* Major, M.C., U. S. Army.

† Captain, M.C., USAR. Present address: Division of Biochemistry, Graduate Hospital of the University of Pennsylvania, Philadelphia 46, Pennsylvania.

METHODS

Glucose or fructose, 10 per cent, 0.5 g/kg, was infused intravenously over a 30-min period into fasting men and dogs. Plasma was sampled at 0, 30, 60, and 120 min. A tungstic acid 1:10 filtrate was prepared and then stored in the frozen state pending analyses. Glucose was determined by the method of Nelson.[22] Plasma amino nitrogen was generally determined by the method of Frame and Russell,[7] and in some instances by a gasometric ninhydrin method modified from Hamilton and Van Slyke.[11] Although the former method is not entirely specific for amino N, serial measure was considered adequate to ascertain gross changes. Plasma tryptophan and phenylalanine were determined on tungstic acid filtrates by a microbiologic technic.[27] The methods employed for laboratory evaluation of liver function have been previously described.[14]

* A media deficient in the specific amino acid was employed to support the growth of *Lactobacillus Arabinosis* (for tryptophan) and *Leuconostoe Mesenteroides* (for phenylalanine). Four increments of each plasma filtrate were tested, each in quadruplicate. Growth proportional to the amount of deficient amino acid added was assayed by titration of the produced lactic acid with a Scholander microburet. The average coefficient of variation of quadruplicate analyses was 3.1 per cent. Recovery of amino acid added to serum was 82 to 108 per cent (mean 94 per cent). The precision of the method was, however, somewhat greater for tryptophan than for phenylalanine due to increased lactic acid production of the test organism under the conditions employed.

A more extensive microbiologic assay of a number of amino acids was originally contemplated as it was thought that small differences in plasma concentration of some amino acids would be obscured by the relatively gross measure of alpha amino nitrogen. However, as microbiologic assay of specific amino acids proved laborious, and was limited by the facilities available, these two amino acids were selected as representative essential components of normal protein synthesis. The "cumulative change" in serial plasma values was determined by plotting the resultant curves of the change from time 0 and determining the area enclosed by each curve.

MATERIAL

(*A*) *Human*

Male patients convalescent from orthopedic surgical procedures served as "normal" controls. All were ambulatory, and eating a regular hospital diet adequate in calories and vitamins. The diet contained an estimated 300 g of carbohydrate per day. Certain additional studies were performed on male laboratory personnel whose diet was not controlled.

Five young male diabetic patients were selected to meet the following criterion: (1) age of onset at age 21 or younger; (2) insulin requirement above 40 units daily; (3) clinical "brittleness" as manifested by a propensity to develop ketoacidosis and/or insulin reactions; (4) stable control for the month preceding study. All were considered compatible with the insulin-deficient type of diabetes.[15] Insulin was omitted on the morning of study.

Two additional patients with anatomic pancreatic deficiency were studied. One was a 46-year-old white male with chronic relapsing pancreatitis. Steatorrhea and moderate diabetes were first noted four years previously. Several surgical procedures attempted for relief of pain were not successful. Extensive calcification of the pancreas was evident. This was confirmed by surgical biopsy two months previous to this study which showed in addition extensive fibrotic and moderate inflammatory changes. A liver biopsy showed nonspecific mild periportal cellular infiltration. Liver function tests were normal.

The second patient was a 38-year-old male in whom a block dissection was carried out two months previously for a carcinoma of the head of the pancreas. Although the pancreatic remnant was anastomosed to the jejunum, progressive glycosuria and ketonuria developed postoperatively. At the time of study, the patient was receiving 30 to 40 units of insulin in divided doses and was taking a liquid diet containing 150 g of carbohydrate as part of his therapeutic regimen. Although he was gaining weight slowly, he was still undernourished and weak. No hepatic metastases were found at exploration, and preoperative liver function studies were normal.

Twenty-four patients with various types of liver injury were selected for study (Table I). Diagnoses were defined by the usual clinical and laboratory criteria, and often confirmed by liver biopsy or surgical exploration. Dietary intake varied as modified by anorexia or dictated by therapy, and was not comparable to the control group. Liver function studies determined at intervals as part of routine workup and management were obtained within several days of the study.

(*B*) *Dogs*

Fasting mongrel dogs of both sexes weighing between 8 and 14 kg were used for these experiments. For at least one month prior to control studies all animals were maintained on a stock kennel diet which supplied 85 g of protein, 45 g of fat, and 140 g of carbohydrate, and was supplemented with vitamins and trace minerals. The choline-deficient diet, modified from Copeland and Salmon,[2,3] was fed from four to six weeks. Although the experimental animals appeared in good condition on the diet, fatty infiltration of the liver (up to 20 per cent of wet weight) was regularly produced. Studies pertinent to the glucose intolerance of some of these dogs have been previously reported from this department.[28]

RESULTS

In the normal subjects plasma amino acids as measured by alpha-amino nitrogen, or by specific assay of phenylalanine and tryptophan were acutely reduced when glucose was in-

TABLE I

Liver Function Studies

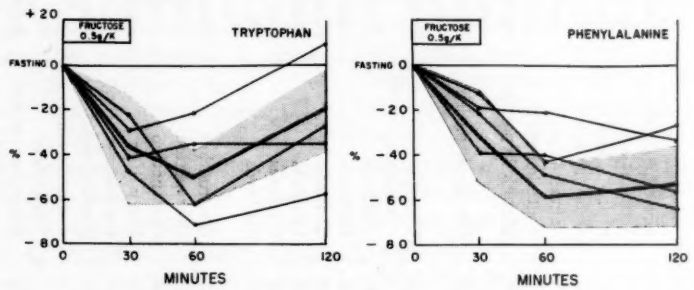
No.	Initial	Age	Diagnosis	Comments	Serum bilirubin *mg/100 ml*		BSP 45 min %	Alkaline phosphatase units	Thymol turbidity units	Zinc turbidity units	24 hr ceph. floc.	Serum protein *g/100 ml*	Serum albumin *g/100 m*
					1 min	Total							
*Normal—upper limit or mean**					*0.25*	*1.0*	*5*	*3.6*	*3.1*	*3.6*	*1+*	*7.4**	*4.3**
1	TF	23	Acute hepatitis	Jaundice 1st week	8.9	18.0	—	—	6.3	6.5	4+	8.8	4.2
2	HM	36	Acute hepatitis	Jaundice 1st week	1.0	3.6	—	—	1.4	2.2	0	6.6	3.4
3	JS	28	Acute hepatitis	Jaundice 1st week	4.3	8.8	—	—	9.8	7.0	3+	7.4	3.4
4	OS	21	Acute hepatitis	Jaundice 2nd week	1.4	2.4	—	10.5	0.6	2.9	0	7.0	3.2
5	K	32	Acute hepatitis	Jaundice 1st week	1.2	2.9	—	—	2.6	4.2	1+	7.8	3.8
6	JC	56	Acute hepatitis	Convalescent 4th week	0.2	0.3	7	—	4.8	6.6	0	7.4	3.2
7	JR	36	Chronic hepatitis	Minimal histologic changes 3 years postacute hepatitis	0.6	1.3	4	6.4	5.1	4.1	0	7.5	3.4
8	AL	27	Chronic hepatitis	Portal fibrosis with inflammatory changes	1.2	2.1	14	2.7	4.7	8.0	1+	6.9	3.0
9	WM	23	Cholangiolytic hepatitis	Surgically explored	0.4	0.8	6	30.0	4.2	5.0	0	7.6	3.8
10	FC	27	Cirrhosis	Alcoholic history	0.1	0.5	9	6.0	1.1	4.0	0	6.3	2.5
11	AB	45	Cirrhosis	Alcoholic history-fatty	0.5	1.3	27	—	2.5	7.3	2+	7.9	3.0
12	WB	51	Cirrhosis	Extensive fatty changes	0.1	1.0	7	3.1	2.7	—	0	7.0	3.9
13	FP	37	Cirrhosis	Postnecrotic	0.2	0.7	19	—	2.1	4.6	0	7.4	3.4
14	SM	42	Cirrhosis	Not proven	0.9	2.2	34	—	0.7	5.5	4+	7.8	2.6
15	HC	26	Cirrhosis	Died in coma several months later	15.8	36.1	—	—	4.4	8.4	4+	8.0	2.7
16	JF	65	Obstruction	Common duct stone	12.5	20	—	20	2.6	—	0	7.4	3.7
17	OP	42	Obstruction	Common duct stone	22.1	45	—	65	3.2	—	1+	7.6	3.8
18	VP	59	Obstruction	Circinoma ampulla of Vater	2.2	4.0	—	16.2	2.3	2.5	0	6.2	2.9
19	JT	31	Fatty liver	Alcoholic history	0.2	0.9	30	2.5	1.7	4.0	0	—	—
20	JK	46	Hemachromatosis		0.1	0.8	16	6.1	2.0	6.1	2+	7.2	3.0
21	AP	24	Familial nonspherocytic jaundice	Normal chromium survival	2.1	2.8	5	4.0	1.8	2.9	—	7.6	3.8
22	PMc	21	Infectious mononucleosis	Jaundice Heterophile + 1:1280	0.4	1.2	—	—	2.1	2.4	1+	7.4	3.8
23	PF	39	Carcinoma of liver	Carcinoma pancreas, metastases	0.2	0.8	24	15	2.3	—	0	6.2	2.5
24	HTM	67	Ca liver	Metastatic, primary site unknown	0.1	0.6	—	24	2.9	2.2	0	7.4	3.4

fused (Table II-A). The maximum depression generally occurred in the 60-min sample and followed the peak in blood sugar. Plasma tryptophan returned almost to fasting values by 120 min; plasma phenylalanine remained depressed. The fasting level of plasma phenylalanine exceeded tryptophan, and the reduction of the former following glucose infusion was somewhat greater. However, no consistent ratio of either absolute concentration of degree of reduction between plasma phenylalanine and tryptophan was apparent. If, however, the cumulative change is compared, the ratio of phenylalanine to tryptophan approximates 1.3, a value not dissimilar from their proportionate growth requirement, and confirms the similar observations of Munro. If fructose rather than glucose is infused, the fall in plasma amino acids is similar although the increment in blood total hexose is much smaller (Fig. 1, Table II-B).

Insulin also induced an acute reduction of plasma amino acids in normal subjects, but the addition of 0.1 U/kilo of regular insulin intravenously either prior to or following the carbohydrate infusion neither aggravated or protracted the usual fall of plasma amino acids (Fig. 2). Similarly, although a standard period of exercise on a treadmill (20 min with belt moving at the rate of approximately 500 feet per min) was shown to reduce plasma α-amino nitrogen by approximately 40 per cent, no summation with the usual response to carbohydrate could be demonstrated (Fig. 3).

The infusion of glucose into the insulin-deficient patient evoked a fall in plasma amino acids in five of the seven subjects (Table III). Although glucose intolerance was evident, the reduction in tryptophan, phenylalanine, and α-amino nitrogen was not dissimilar from that in the normal group. An irregular response was evident in one spontaneous diabetic (Pt. AA) and in the surgical patient with a partial pancreatectomy (Pt. JA). The former was seemingly similar to the other diabetic patients in regard to the clinical severity of his diabetes. It was subsequently observed, however, that he had a palpably enlarged liver and mild sulfobromophthalin (BSP) retention. The surgical patient with the paradoxic response to glucose was subsequently found at autopsy two months later to have massive fatty infiltration of the liver. It is likely, therefore, that liver function was disturbed at the time of study. However, it was of interest that a dose of insulin sufficient to effect a severe hypoglycemia in this patient was similarly ineffective in lowering blood amino nitrogen. It seemed possible, therefore, that modification or mitigation of the usual fall in plasma amino acids associated with increased carbohydrate utilization might be found in other patients with liver injury.

The effect of glucose loading in patients with liver disease is shown in Table IV. The usual



% CHANGES IN PLASMAS TRYPTOPHAN AND PHENYLALANINE IN NORMAL SUBJECTS RECEIVING FRUCTOSE.
MEANS CHANGES ±SD AFTER GLUCOSE.

Fig. 1. The effect of fructose on plasma tryptophan and phenylalanine in normal men. The shaded area represents for comparison the changes resulting from a similar infusion of glucose.

TABLE II

Effect of Carbohydrate Infusion in Normal Subjects

Minutes	Tryptophan mg/liter				Phenylalanine mg/liter				Glucose mg/100 ml				Amino N mg/100 ml			
	0	30	60	120	0	30	60	120	0	30	60	120	0	30	60	120
A. Normals																
Glucose 1	10.0	8.1	6.1	6.5	12.4	13.8	6.1	5.1	64	218	132	62	4.6	4.3	4.0	4.1
0.5 g/kg 2	7.8	3.1	2.7	4.5	15.1	8.2	4.4	5.0	90	185	126	69	8.1	7.9	5.8	6.9
3	10.2	10.2	6.8	4.4	14.6	9.0	6.9	4.2	86	205	135	78	6.0	6.4	4.9	4.3
4	9.3	6.7	4.3	7.1	16.0	11.3	6.0	6.5	84	168	118	72	5.9	5.7	5.6	5.3
5	9.6	7.7	4.7	11.3	11.6	9.2	7.7	11.2	92	174	137	84	5.7	5.2	5.0	5.4
6	10.1	5.6	5.3	7.4	11.4	4.3	3.4	6.2	77	242	145	90	6.1	4.8	5.2	5.9
7	8.9	3.1	3.9	6.6	12.3	10.2	8.1	8.4	63	185	94	67	6.6	6.0	6.0	6.3
8	12.0	4.4	5.5	14.7	13.2	6.0	2.6	4.3	88	204	103	82	7.3	7.0	6.9	7.0
Mean	9.74	6.11	4.91	7.81	13.32	9.00	5.65	6.36	80.5	197.0	123.8	75.5	6.28	5.91	5.42	5.65
+ S. D.	1.09	2.39	1.22	1.82	1.64	2.77	1.87	2.24	10.7	23.9	16.5	8.9	1.04	1.12	0.84	1.01
B. Normals																
Fructose 1	10.2	5.4	2.9	4.3	11.4	8.6	7.4	6.7	84	126	94	76	—	—	—	—
0.5 g/kg 3	11.3	8.7	4.3	8.2	14.6	9.2	9.0	6.8	—	—	—	—	—	—	—	—
9	10.0	5.8	6.4	6.5	13.2	10.3	6.3	4.2	65	88	124	76	6.9	6.8	5.4	5.7
10	10.4	7.3	8.2	11.4	14.1	13.0	8.1	10.4	90	146	130	60	4.8	4.2	4.2	4.0

TABLE III

Effect of Carbohydrate Infusion in Subjects with Presumptive Insulin Deficiency

Minutes	Tryptophan mg/liter				Phenylalanine mg/liter				Glucose mg/100 ml				Amino N mg/100 ml			
	0	30	60	120	0	30	60	120	0	30	60	120	0	30	60	120
Diabetes mellitus																
SJ	10.7	8.3	6.5	6.3	14.3	6.2	5.8	8.4	120	242	210	164	—	—	—	—
LT	10.9	6.4	8.2	10.0	13.6	9.2	9.8	10.2	210	308	lost	260	—	—	—	—
Glucose LR	8.3	4.9	6.3	11.3	11.6	8.1	9.0	9.3	160	—	—	110	5.2	3.2	4.6	4.4
0.5 g/kg SW	11.4	10.0	8.3	7.9	14.3	4.3	5.9	5.8	140	—	—	164	4.6	3.8	3.6	3.8
AA	8.9	9.2	9.9	10.3	12.4	13.2	16.0	14.7	90	184	168	120	6.3	3.8	3.6	3.8
Chronic pancreatitis																
CK	10.7	6.2	5.8	6.3	16.2	8.8	11.6	10.8	160	306	274	140	6.0	6.4	4.2	6.8
Pancreatectomy*																
JA	7.2	14.2	11.6	10.4	10.4	11.2	13.1	12.0	62	294	242	164	4.3	5.4	5.2	6.0

* Abnormal response.

diminution of plasma tryptophan and phenylalanine following glucose infusion was grossly deranged in six patients, and less than normal in six others. Thus, half of a diverse group of patients with liver disease did not respond normally. In general these were patients with

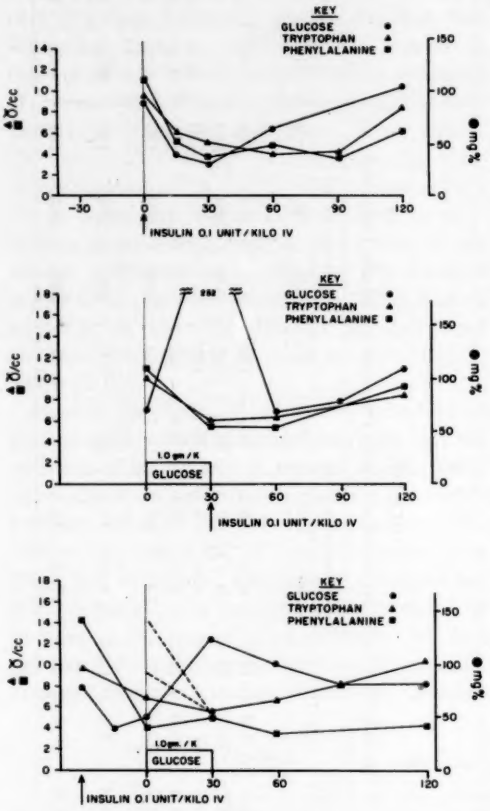


Fig. 2. The effect of insulin on a normal subject. Although insulin depressed plasma tryptophan and phenylalanine (top), the effect of glucose was neither exaggerated or protracted when insulin was given following (middle) or preceding (lower) the carbohydrate infusion. In other subjects the insulin effect on amino acids was somewhat shorter in duration than in the subject depicted. The subject is the same individual as normal 7 in Table II.

cirrhosis or acute hepataitis with severe abnormalities evident from liver function tests, and most were clinically jaundiced. Yet not all the patients with severe liver disease showed a definitely abnormal response, and all four

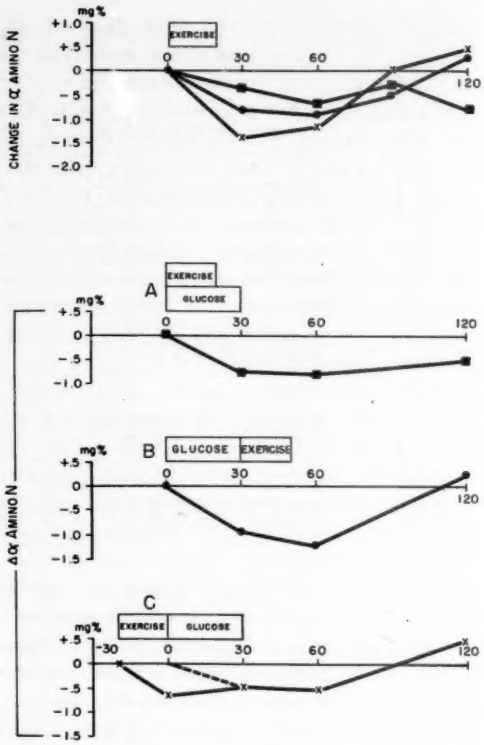


Fig. 3. The effect of exercise in three normal subjects. Each subject was studies during an exercise period and during a combination of exercise and glucose infusion. Whether subject exercised during (A), after (B), or before (C) the glucose infusion, the resultant fall in α-amino nitrogen was similar to that produced by glucose alone. The subject of experiment C is the same individual as normal 2 in Table II. Blood glucose was little changed by exercise alone, but fell somewhat more rapidly when exercise was combined with glucose infusion. Subject of experiment B complained of hypoglycemic symptoms at 60 minutes when blood sugar was 64 mg per 100 ml.

patients with obstructive jaundice responded normally (patients 9, 16, 17, 18).

Generally plasma tryptophan and phenylalanine were affected similarly, although there was some suggestion that the response of the latter was abnormal when the plasma tryptophan response was normal or equivocal (patients 11 and 20). This had the effect of disrupting any constant ratio between the two amino acids. Although the fasting values of both amino acids were on occasion without the 95 per cent

TABLE IV

The Effect of Glucose Infusion in Patients with Liver Disease

	Tryptophan mg/liter				Phenylalanine mg/liter			
	0	30	60	120	0	30	60	120
Normal mean	9.7	6.1	4.9	7.8	13.3	9.0	5.6	6.4
S. D.	1.09	2.39	1.22	1.82	1.64	2.77	1.87	2.24
Case no. 1*	8.9	9.3	15.0	12.8	14.0	17.0	23.0	13.0
2*	5.2	6.1	6.4	8.6	10.5	12.2	9.0	8.6
3†	7.0	7.0	7.5	8.2	13.6	10.0	10.1	10.6
4	6.3	3.4	7.5	12.2	—	—	—	—
5*	8.7	6.0	10.0	14.3	—	—	—	—
6	9.0	3.1	4.4	7.7	18.9	13.4	7.4	8.4
7	10.0	7.2	5.0	9.6	14.5	10.5	10.0	9.8
8	11.2	8.9	7.0	14.1	13.4	12.8	12.2	14.7
9	14.5	9.6	6.3	16.0	—	—	—	—
10†	9.0	8.5	7.6	7.9	13.4	10.1	10.2	11.1
11†	9.0	9.1	7.0	8.0	10.0	15.0	10.5	10.1
12†	21.3	13.9	14.6	12.8	—	—	—	—
13†	11.5	14.0	10.0	15.0	20.0	20.5	14.5	23.0
14*	6.5	10.5	8.7	12.1	13.0	12.7	14.2	17.6
15*	16.1	18.2	19.9	30.7	—	—	—	—
16	10.5	8.3	6.1	7.8	11.1	8.2	5.0	7.1
17	11.2	5.7	6.0	9.2	14.6	8.6	6.1	6.5
18	10.1	7.2	5.7	8.1	12.0	9.3	7.4	6.0
19	8.9	10.9	12.8	10.4	12.2	13.0	12.5	16.6
20	10.1	8.6	6.4	8.9	16.2	15.4	15.0	14.6
21	11.2	—	8.7	8.9	—	—	—	—
22†	14.0	13.7	12.8	13.1	—	—	—	—
23	8.6	8.5	6.1	8.9	—	—	—	—
24	9.6	7.2	5.4	8.3	15.0	10.4	9.3	8.7

* Abnormal response.

† Possibly abnormal response.

limits of the normal group, it is likely that the two populations are not validly compared due to differences in age, nutritional status, diet, and other uncontrolled factors. No relationship between fasting level, and the amino acid response to glucose infusion was apparent. Furthermore, there was no apparent correlation of the magnitude of the abnormal response and any single laboratory test of liver function although the material presented is insufficient for adequate analysis.

Dog Experiments

The response of plasma amino acids to carbohydrate infusion in normal and choline-deficient dogs is shown in Table V. In no animal with fatty liver was the pattern of response normal (group III). The abnormal response, however, could not be quantitatively correlated with fat content of the liver, or liver test abnormalities. The response in normal dogs made hyperglycemic by increasing the glucose load fourfold was essentially normal (group IV).

DISCUSSION

The fall in plasma amino acids does not appear to bear any direct relationship to the blood sugar level other than to the increased rate of carbohydrate utilization presumably induced by hyperglycemia. If one explains this phenomenon as evidence that some product of carbohydrate utilization serves to increase the net synthesis of protein, and thereby reduces the plasma pool, it is difficult to explain the temporary nature of this reduction. The rapid return to normal in the fasting subject would suggest that although plasma amino acids may be an immediate precursor of cellular proteins, some less readily available source of essential amino acids subsequently repletes the plasma

TABLE V

Effect of Glucose Infusion on Plasma Phenylalanine and Tryptophan of Normal and Choline-Deficient Dogs

		Weight by	Sex	Infused	Tryptophan mg/liter				Phenylalanine mg/liter				Glucose mg/100 ml			
					0	30	60	120	0	30	60	120	0	30	60	120
I	Normal	10.0	F	Glucose 0.5 g/kg	8.0	5.0	2.0	8.1	12.1	5.5	4.5	12.3	99	175	110	85
	Normal	13.6	M	"	12.5	10.0	6.8	5.3	13.2	5.2	4.3	8.6	84	179	121	95
	Normal	10.7	F	"	14.3	10.2	8.7	11.9	15.2	6.9	5.8	9.3	72	—	—	90
	Normal	9.3	F	"	12.0	5.0	2.1	8.1	14.1	7.4	6.9	6.3	84	—	—	76
	Normal	12.7	M	"	11.3	4.0	1.8	3.3	11.6	3.0	9.6	5.3	110	165	115	102
	Normal	8.2	F	"	10.4	8.3	5.2	7.9	15.3	8.9	6.3	8.4	88	—	—	94
				Normal												
II	Normal	15.0	F	Saline 100 ml	9.7	10.1	9.4	9.5	12.1	12.5	11.1	11.0	85	79	84	92
	Normal	9.0	M	"	12.6	12.4	13.1	10.9	13.9	12.1	12.4	12.6	80	86	86	74
III	Choline-deficient	11.0	M	Glucose 0.5 g/kg	6.2	11.5	8.1	9.0	8.5	6.6	5.8	6.0	67	187	131	84
	Choline-deficient	11.0	F	"	9.0	11.0	6.9	15.6	12.5	8.5	9.5	14.0	114	245	182	153
	Choline-deficient	9.1	F	"	10.0	8.5	8.5	8.7	12.7	12.0	11.6	11.5	77	235	168	167
	Choline-deficient	12.3	F	"	6.0	7.9	12.8	11.5	7.4	9.5	11.6	13.3	126	240	194	141
	Choline-deficient	14.1	M	"	10.3	11.2	11.0	9.5	16.2	14.1	16.3	17.2	160	310	245	182
	Choline-deficient	8.2	F	"	8.7	8.3	8.3	9.2	9.7	9.9	10.1	8.2	84	190	161	102
	Mean															
	100															
IV	Normal	10.0	F	Glucose 2.0 g/kg	12.3	4.6	3.8	3.3	12.6	4.0	7.6	8.3	70	465	276	154
	Normal	15.4	F	"	16.3	11.2	11.7	13.9	12.2	5.5	4.8	8.3	74	642	450	285
	Normal	8.7	F	"	9.6	4.3	5.1	5.9	18.2	11.8	8.0	6.4	74	500	155	82
	Normal	11.1	F	"	10.1	2.2	4.2	6.2	13.0	8.3	9.5	11.0	lost	526	204	140

pool. As plasma amino acids are reduced when glucose is given to the insulin-deficient men, and inasmuch as fructose, which is presumably to some degree independent of insulin for its utilization,[19] is equally effective in reducing plasma amino acids, the unessentiality of insulin action is suggested. It would appear that insulin induces the fall of plasma amino acids solely by increasing the rate of glucose utilization. Once a maximum fall in amino acids is effected, further increment of carbohydrate utilization, whether produced by further increasing blood sugar, by supplementation with exogenous insulin, or by exercise, may protract but not exaggerate this effect. This too is reminiscent of the protein-sparing activity of carbohydrate in the fasting subject, whereby once a minimum of carbohydrate is fed, further increments of dietary carbohydrate are progressively less effective as nitrogen-sparers.[9]

A normal functioning liver would appear a requisite for the usual fall in amino acids resulting from carbohydrate infusions. The fasting level of plasma amino acids is usually maintained with great constancy, and in spite of even extensive liver injury may remain normal until hepatic failure is imminent.[8,10,17,23,29] Then not only is the total as measured by amino nitrogen content increased but the pattern and proportions of the amino acids present may be distorted.[13,29] The abnormal amino acid response to carbohydrate loading suggests, however, that alteration in amino acid metabolism may attend hepatic dysfunction without alteration of fasting levels. Although glucose tolerance was measured in only a few of the patients, it is likely that the abnormal glucose tolerance evident in certain instances of both the experimentally induced and the observed liver disease is not directly related to the amino acid response, as shown by the normal response in the human diabetics, and in the normal dogs receiving four times the usual dose of glucose. If it is assumed that fatty infiltration of the liver or some feature of lipotrophic deficiency affects amino acid metabolism directly, the abnormal response in three of six instances of severe acute hepatitis remains unexplained. Similarly quantitative correlation is lacking with evidence of disturbed protein synthesis as reflected in hypoalbuminemia and hyperglobulinemia.

It is likely that the reduced concentration of plasma amino acids stems from an acute alteration in carbohydrate metabolism in the liver, a site where insulin may be of lesser importance. Increased hepatic utilization of glucose may increase the availability of tricarboxylic acid intermediates which may in turn serve as acceptors of amino nitrogen. Thus, when glucose is infused into fasting men, the equilibrium of the transaminase reactions involving α-ketoglutaric, oxalacetic and pyruvic acid is shifted so that more amino acids are removed from the plasma as the result of intrahepatic accumulation of more acceptors of amino groups. While aspartic and glutamic acid, and alanine are considered to be directly conceived in transamination reactions it is probable that other amino acids may also be indirectly involved resulting temporarily in a net shift of α-amino nitrogen from the plasma to the liver.

Although a relatively constant relationship is evident between plasma tryptophan and phenylalanine in the normal men and dogs, the dissociation of this relationship in several cases of patients with liver disease and in some of the choline-deficient dogs would cast doubt on the concept that the reduction of amino acids following increased carbohydrate utilization is related to protein sparing or involves changes involving the entire protein molecule. It is consistent, however, with our observations that plasma tryptophan may be reduced while plasma phenylalanine is normal or slightly elevated in acute hepatitis.[24]

Both cases of obstructive jaundice due to stone, the case of carcinoma of the ampulla of Vater and the single case of cholangiolytic hepatitis, showed a normal amino acid response despite moderately severe jaundice. While insufficient material has been studied, it is possible that the amino acid response to carbohydrate loading may be useful in certain cases in the differential diagnosis of hepatocellular vs. surgical jaundice.

SUMMARY

Plasma amino acids are reduced when glucose

or fructose are infused into fasting normal men and dogs. Insulin or exercise, which by themselves lower plasma amino acids, do not further decrease plasma amino acids when superimposed upon carbohydrate infusion. Diabetic patients selected as likely to be insulin-deficient and normal dogs given large amounts of glucose to produce hyperglycemia demonstrated a normal fall. It is suggested that the fall in plasma amino acids is related to the increased rate of carbohydrate utilization and is largely independent of the level of blood sugar or the action of insulin. Choline-deficient dogs with fatty liver and approximately one-half of a diverse group of patients with liver disease responded abnormally.

These data indicate that intact hepatocellular function is necessary for the reduction of plasma amino acids following carbohydrate infusion and suggest that the liver is a major site of removal of amino acids from the plasma under these circumstances.

ACKNOWLEDGMENTS

The authors greatly appreciate the advice and encouragement offered by Dr. Victor Sborov. Drs. H. L. Smith, Burton Giges, and Richard Ettinger initiated the studies of carbohydrate tolerance in patients and choline-deficient dogs which prompted the reported observations. Mr. Amos Lewis assisted in the tabulation and organization of the data.

REFERENCES

1. Albanese, A. A., Orto, L., Rossy, J., Dihallo, R., and Belmont, A.: Effect of carbohydrates on blood amino nitrogen. *Metabolism* 4: 160, 1955.
2. Copeland, D. H. and Salmon, W. D.: The occurrence of neoplasms in the liver, lungs, and other tissues of rats as a result of prolonged choline deficiency. *Am. J. Path.* 22: 1059, 1946.
3. Engel, R. W. and Salmon, W. D.: Improved diets for nutritional and pathologic studies of choline deficiency in young rats. *J. Nutrition* 22: 109, 1941.
4. Flock, E. V., Block, M. A., Mann, F. C., Gridley, J. H., and Bollman, J. L.: Effect of glucose on the amino acids of plasma after total hepatectomy. *J. Biol. Chem.* 198: 427, 1952.
5. Folin, O. and Berglund, H.: The retention and distribution of amino acids with especial reference to urea formation. *J. Biol. Chem.* 51: 395, 1922.
6. Frame, E. G. and Russell, J. A.: The effects of insulin and anterior pituitary extract on the blood amino nitrogen in eviscerated rats. *Endocrinology* 39: 420, 1946.
7. Frame, E. G., Russell, J. A., and Welhelmi, A. E.: The colorimetric estimation of amino nitrogen in blood. *J. Biol. Chem.* 149: 255, 1943.
8. Gabuzda, G. J., Eckhardt, R. D., and Davidson, C. S.: Urinary excretion of amino acids in patients with cirrhosis of the liver and in normal adults. *J. Clin. Investigation* 31: 1015, 1952.
9. Gamble, J. L., Ross, G. S., and Tisdall, F. F.: Metabolism of fixed base during fasting. *J. Biol. Chem.* 57: 663, 1923.
10. Greene, C. H., Sandeford, K., and Ross, H.: The amino acid content of the blood in normal and pathologic conditions. *J. Biol. Chem.* 58: 845, 1923–24.
11. Hamilton, P. B. and Van Slyke, D. D.: The gasometric determination of free amino acids in blood filtrated by the ninhydrin-carbon dioxide method. *J. Biol. Chem.* 150: 231, 1943.
12. Harris, M. M. and Harris, R. S.: Effect of insulin hypoglycemia and glucose on various amino acids in blood of mental patients. *Proc. Soc. Exper. Biol. & Med.* 64: 471, 1947.
13. Iber, F. L., Rosen, H., Levenson, S. M., and Chalmers, T. C.: Plasma amino acids in patients with liver failure. *J. Lab. & Clin. Med.* 50: 417, 1957.
14. Knowlton, M., Horner, W. H., Seligson, D., and Iber, F. L.: *Analytical Procedures Laboratory Manual.* Army Medical Service Graduate School, Walter Reed Army Medical Center, August, 1955.
15. Lawrence, R. D.: Three types of human diabetes. *Ann. Int. Med.* 43: 1199, 1955.
16. Luck, J. M. and Morse, S. W.: Effects of insulin and adrenaline on the amino acid content of blood. *Biochem. J.* 27: 1648, 1933.
17. Mann, F. C.: The effects of complete and of partial removal of the liver. *Medicine* 6: 419, 1927.
18. Milman, A. E., DeMoor, P., and Lukens, F. D. W.: Relationship of purified pituitary growth hormone and insulin in regulation of nitrogen balance. *Am. J. Physiol.* 166: 354, 1951.
19. Miller, M., Drucker, W. R., Owens, J. E., Craig, J. W., and Woodward, H., Jr.: Metabolism of intravenous fructose and glucose in normal and diabetic subjects. *J. Clin. Investigation* 31: 115, 1952.
20. Munro, H. N.: Carbohydrate and fat as factors in protein utilization and metabolism. *Physiol. Rev.* 31: 449, 1951.
21. Munro, H. N. and Thomson, W. S. T.: Influence of glucose on amino acid. *Metabolism* 2: 354, 1953.
22. Nelson, N.: A photometric adaptation of the Somogyi method for the determination of glucose. *J. Biol. Chem.* 153: 375, 1944.

23. PETERS, J. P. and VAN SLYKE, D. D.: *Quantitative Clinical Chemistry.* Williams and Wilkins, Baltimore, 1946, pp. 675 and 806.

24. RUBINI, M. E. and SELIGSON, D.: Unpublished observations.

25. RUSSELL, J. A.: Hormonal control of amino acid metabolism. *Fed. Proc.* 14: 696, 1955.

26. SCHMIDT, E. G. and EASTLAND, J. S.: The influence of dextrose ingestion on amino acid nitrogen, urea nitrogen, and hemoglobin concentration of the blood. *J. Lab. & Clin. Med.* 21: 1, 1935.

27. SELIGSON, D., TORGHELE, R. J., and ANDERSON, J. A.: An improved method for the determination of amino acids in small samples of blood and urine. *J. Lab. & Clin. Med.* 35: 640, 1950.

28. WALDSTEIN, S. S., ETTINGER, R. H., and GIGES, B.: Abnormal glucose tolerance in experimental choline deficient fatty liver. *Metabolism* 6: 134, 1957.

29. WALSHE, J. M.: Disturbances of amino acid metabolism following liver injury: A study by means of paper chromatography. *Quart. J. Med.* (N.S.) 22: 483, 1953.

The Influence of Maternal Iron Deficiency on the Newborn

THOMAS R. C. SISSON, M.D.* AND CURTIS J. LUND, M.D.†

THE DEVELOPMENT of hypochromic microcytic anemia in later infancy is not believed to result solely from nutritional iron deficiency. A depletion of iron stores in early infancy, amplified by the demands of rapid growth, is a pre-eminent factor.

The purpose of this investigation was to study the pathogenesis of anemia in the first few months of life by exploring the possibility that iron deficiency in the mother is reflected in the newborn infant.

The significance of maternal iron in the production of the anemia of infancy has been debated for many years. Strauss[1] concluded that anemia in the pregnant woman would lead to anemia in her infant sometime during the first year of life. Woodruff and Bridgeforth,[2] on the other hand, were unable to demonstrate a relation between the mother's hemogram during pregnancy and that of her infant. Nor did Oettinger, Mills, and Hahn[3] find a relationship between the mother's hemoglobin at the time of birth and the amount of iron absorbed by the infant. Woodruff[4,5] in later publications demonstrated that anemia is more common and severe in infants of low birth weight and high birth order. Similar findings have been reported by Guest and Brown.[6] These reports serve to confirm the generally held impression that several factors influence the anemia of infancy, and that some maternal factors are of profound importance.

The assessment of relative states of iron deficiency, especially in pregnant women, has been controversial. It has been apparent that in certain circumstances measurement of the total circulating hemoglobin mass and red cell volume gives a more reliable index of an anemic state than the red cell count and hemoglobin concentration. Standards for the laboratory diagnosis of anemia depend, for accuracy, on the constancy of the ratio of hemoglobin and red cell mass to plasma volume and body size. More precise definition is possible by observing biochemical changes in the hematopoietic system. These are most helpful in the study of pregnant women and their infants because of the striking volumetric changes which commonly occur, and which account in great part for the seeming contradictions in the observations already cited.[1-3]

We have found that in a sizable number of pregnant women the simple measurement of hemoglobin concentration and packed red cell volume is not trustworthy in diagnosing anemia because of states of hypo- and hypervolemia.[7]

For these reasons we have relied on measurement of red cell volume and circulating hemoglobin mass to determine the hematologic standing of the subjects of the investigation. These volumetric studies have served to appraise quantitatively the influence of maternal iron deficiency on the newborn infant.

METHODS

The standards set in this laboratory as minimum normal values for the non-anemic pregnant woman are:

Red cell volume, 30.0 ml/kg
Hemoglobin mass, 11.0 g/kg

From the Departments of Pediatrics and Obstetrics and Gynecology of the University of Rochester School of Medicine and Dentistry, Rochester, New York.

* Instructor in Pediatrics and Instructor in Obstetrics and Gynecology.

† Professor and Chairman of the Department of Obstetrics and Gynecology.

Read at the annual meeting of The Society for Pediatric Research, Carmel, California, June 21, 1957.

Supported by Grant A-515 (C) from the National Institute of Arthritis and Metabolic Diseases of the United States Public Health Service.

Sixty-six women were studied throughout pregnancy, and their infants were studied in the first three to five days of life (after shifts in the plasma compartment had stabilized). The pregnancies were normal except for the presence of anemia in some of the mothers. All deliveries were spontaneous and without anesthesia unless pudendal block anesthesia was employed. The infants were normal, full-term, and without neonatal complications. No infant was included who exhibited hemolytic disease of the newborn or other hematologic disorder.

The diagnosis of maternal iron deficiency was made from values established in the last half of the third trimester of pregnancy. The mothers were between the ages of 20 and 35 years and of various parity. The birth weights of the infants ranged from 2,500 to 4,090 gr. No infant was included of less than 39 weeks' gestation.

The mothers were divided into three groups: *non-anemic*, whose hemoglobin mass was greater than 11.0 g/kg; *intermediate* or *moderately anemic* whose mass was between 9.5 and 11.0 g/kg; and *severely anemic*, whose hemoglobin mass was below 9.5 g/kg. Each infant was automatically placed in his mother's group and designated in the same fashion for simplicity's sake. There were 20 mothers plus their babies in the non-anemic class, 21 in the "intermediate," and 25 in the "severely anemic" group.

Laboratory examinations were done by micro or semimicro technics because of the necessity of duplicating the entire battery of studies in the newborn infants. A total of 3.5 to 4.0 ml of blood was drawn for each study from an antecubital vein in the mother, and from a femoral vein in the infant. Dye injections were performed through an antecubital vein in both woman and infant.

Hemoglobin concentration was measured by the cyanmethemoglobin method of Crosby, Munn, and Furth.[8] Microhematocrits were determined by the use of an International Hemocrit Centrifuge.® Plasma volume was measured by a slight modification of the method of Nitsche and Cohen[9] using T-1824 or Evans Blue, and by elution of the dye in acetone-water as described by Allen.[10] Recovery experiments yielded 97.0 ± 2.0 per cent dye on samples in triplicate. From these measurements were derived the values for total blood volume, total red cell volume, and total circulating hemoglobin mass. In these calculations the factors for plasma trapped in the hematocrit tube,[11,12] and for the ratio of body/venous hematocrit[13] were used. Total circulating hemoglobin mass was calculated from the total blood volume and hemoglobin concentration. Volume and mass determinations (except where noted) are reported on the basis of body weight since such a variation of body size existed in the subjects. Serum iron was measured by the method of Burch, Lowry, and Bessey,[14] and latent serum iron-binding capacity by a slight modification of the method of Rath and Finch.[15] Red cell count was done by the same experienced technologist throughout the investigation using accepted methods for hematologic study.

RESULTS

A compilation of the laboratory results are given in Tables I, II, and III. Mean values for total members of each group, and mean values for infants of treated and untreated mothers* are listed in Table IV.

There is considerable disparity between the values for hemoglobin concentration and hemoglobin mass as well as between the values for hematocrit and red cell volume in the mothers. The absolute differences in the groups are more conspicuous if the latter values are considered than if the hemoglobin concentrations and hematocrit readings are compared. The paradoxic finding that some women with low hemoglobin concentrations have high hemoglobin mass values and vice versa is explained by the differences in blood volume. Further studies and an exposition of these observations will appear in another report.[7]

* The mothers were selected from subjects of a longitudinal survey of oral iron therapy in pregnancy, wherein a "blind" study was done.

Ferrous sulfate or molybdenized ferrous sulfate in a dosage of 600 mg daily by mouth was begun during the second trimester of pregnancy and maintained until term. The actual intake and absorption could not be verified in all subjects thus treated on an outpatient basis. The lack of therapeutic response in some patients suggests that in at least a few cases the intake or absorption of iron may have been inadequate.

(A.) *Hemoglobin Concentration:* The difference in hemoglobin concentrations between the infants of non-anemic and severely anemic mothers is 1.1 g/100 ml, and between those of moderately anemic and severely anemic mothers 0.8 g/100 ml. These differences are not significant. The range of values in each group of infants is similar.

No significant differences existed between the packed red cell volumes of the three groups, and the range of values, though not exactly equal, was nearly so.

(B.) *Plasma Volume:* The mean plasma volume of all infants in the series was 61.0 ml/kg. That of the non-anemic group was 65.6 ml/kg, that of the moderately anemic group was 60.8 ml/kg, and the mean of the severely anemic group was 58.2 ml/kg. It was noted that the range of values, though approximately equal in all groups, was at a higher level in the non-anemic infants.

States of hypo- and hypervolemia occur in the newborn and older infant[16] and in the premature.[17] Five of the infants in this study were considered to be hypovolemic, having a plasma volume below 45 ml/kg; and ten infants were hypervolemic, having a plasma volume greater than 75 ml/kg. A distribution of individual volumes is illustrated in Figure 1.

(C.) *Total Blood Volume:* The mean blood volume of infants in the non-anemic group was 111.7 ml/kg. Of these infants, eight were babies of women who had been treated with oral iron during pregnancy, and 12 were babies of untreated women. The mean blood volume of the first eight infants was 110.9 ml/kg, and of the other 12 the mean was 112.3 ml/kg. These values may be considered equal, and they are significantly higher than values for infants of untreated anemic women.

The mean blood volume of the moderately anemic group of infants was 103.2 ml/kg. The

TABLE I

Infants of Non-Anemic Mothers

Name	Sex	Rx of mother	Birth weight g	Hgb g %	PRCV %	Plasma volume ml/kg	Blood volume ml/kg	Red cell volume ml/kg	Hb Mass g/kg	Hb Mass g	Serum γ %	Iron γ/kg
Atk	M	$FeSO_4$	3,880	15.8	48.0	43.5	72.1	28.6	11.4	42.2	78	34
Bro	M	None	3,120	17.7	51.0	59.0	102.0	42.9	18.1	54.2	59	35
Jen	M	None	3,000	12.6	37.0	75.7	109.0	33.3	13.8	41.8	50	38
Car	M	None	3,380	16.3	46.0	73.5	118.2	45.0	19.3	65.3	46	34
Gar	M	$FeSO_4$	3,000	17.2	51.0	65.2	112.8	47.6	19.4	56.3	153	100
Hal	F	None	3,470	19.2	57.0	62.1	117.4	55.3	22.6	75.8	106	66
Jop	M	None	2,900	17.5	51.0	66.5	114.9	48.5	20.1	57.3	111	74
Kee	F	None	2,650	18.0	49.0	54.9	92.3	37.4	16.6	42.2	98	54
Mul	M	Mol Iron	3,750	18.3	56.0	49.4	92.0	42.6	16.8	59.1	81	40
Phe	M	$FeSO_4$	3,480	13.2	39.0	69.2	102.1	32.9	13.5	46.6	86	60
San	F	None	3,450	17.0	51.0	66.5	115.0	48.5	19.6	65.3	100	67
Smi	M	None	3,860	16.2	45.0	63.1	100.5	37.4	16.3	62.2	100	63
Sut	M	None	3,700	16.4	56.0	62.3	116.0	53.7	19.0	68.5	33	21
Tri	F	None	2,810	16.2	45.0	63.5	101.2	37.6	16.4	43.6	111	71
Woo	M	None	2,950	17.5	52.5	83.9	148.2	64.3	25.9	70.3	50	42
Gol	F	None	3,000	15.0	49.0	67.0	112.7	45.6	16.9	48.5	80	54
Ale	M	$FeSO_4$	3,700	16.8	54.0	71.2	128.6	57.4	21.6	79.3	103	73
Bro	F	$FeSO_4$	3,480	19.4	56.0	62.8	116.8	54.1	22.7	76.4	162	102
Con	M	Mol Iron	2,600	16.2	48.0	67.5	111.8	44.3	18.1	48.4	—	—
Nud	F	$FeSO_4$	4,100	19.6	53.0	85.1	151.3	66.3	29.7	121.3	153	130
Mean			3,314	16.8	49.7	65.6	111.7	46.2	18.9	61.2	93	61
Median			3,415	16.9	51.0	66.0	112.8	45.3	18.5	58.2	98	60
Means of:												
8 treated			3,490	17.1	50.6	64.2	110.9	46.7	19.2	66.2	117	77
12 untreated			3,208	16.6	49.1	66.5	112.3	45.8	18.7	57.9	79	52

TABLE II

Infants of Intermediate Group of Mothers

Name	Sex	Rx of mother	Birth weight g	Hgb g %	PRCV %	Plasma volume ml/kg	Blood volume ml/kg	Red cell volume ml/kg	Hb Mass g/kg	Hb Mass g	Serum Iron γ %	Serum Iron γ/kg
Gon	M	None	3,080	18.2	58.0	60.9	117.0	56.1	21.3	63.9	66	40
All	M	$FeSO_4$	3,700	13.4	42.0	87.6	134.7	46.9	18.0	67.5	111	97
Ban	M	Mol Iron	3,120	15.5	43.5	68.9	107.6	38.7	16.9	49.2	100	69
Dav	M	None	3,460	16.2	48.5	58.6	97.7	39.2	15.8	52.6	61	36
Dav	F	None	2,900	15.5	51.0	56.6	97.9	41.2	15.2	43.8	72	41
Dro	F	$FeSO_4$	3,170	14.5	47.0	86.6	141.6	55.0	20.5	64.9	139	120
Fer	F	$FeSO_4$	3,840	17.1	54.0	54.7	98.8	44.1	16.9	60.3	55	30
Fra	F	None	3,360	15.3	46.0	76.6	123.7	47.0	18.9	63.6	58	44
Fra	M	$FeSO_4$	2,850	15.6	46.0	45.0	72.6	27.6	11.3	33.6	81	37
Har	M	$FeSO_4$	2,830	15.4	49.0	46.7	78.4	31.8	12.1	31.9	108	50
Hob	F	None	3,320	18.8	58.5	51.6	99.9	48.3	18.8	59.7	82	42
Ive	F	None	3,550	16.7	47.0	78.6	128.6	49.9	21.5	74.1	66	52
Lin	M	None	2,770	18.0	51.0	61.9	107.0	45.1	19.3	52.8	78	48
Mad	F	None	2,780	18.4	56.5	55.5	104.2	48.7	19.2	53.3	92	51
Smi	M	None	3,140	15.6	48.0	44.9	74.4	29.5	11.6	37.8	72	32
StC	F	$FeSO_4$	2,880	17.1	50.0	58.8	100.2	41.4	17.1	45.9	184	108
Mou	M	None	3,600	18.9	58.0	36.3	69.8	33.5	13.2	45.6	25	9
Pri	M	None	4,360	13.4	40.5	63.0	94.7	31.7	12.7	54.5	81	51
Rog	M	None	3,720	19.2	55.0	46.0	84.4	38.4	16.2	60.3	76	35
Smi	M	$FeSO_4$	2,880	16.8	50.0	47.4	80.8	33.4	13.6	38.9	56	27
Tay	F	None	3,250	17.1	50.0	89.6	152.6	63.1	26.1	82.2	106	95
Mean			3,265	16.5	50.0	60.8	103.2	42.4	16.9	54.1	84	53
Median			3,170	16.7	50.0	58.6	99.9	41.4	16.9	53.3	78	44
Means of:												
8 treated			3,159	15.7	47.8	62.0	101.8	39.9	15.8	49.9	104	67
13 untreated			3,330	17.0	51.4	60.0	104.0	44.0	17.7	57.2	72	44

means for eight babies of treated and 13 babies of untreated mothers was 101.8 ml/kg and 104.0 ml/kg, respectively.

There was a distinct difference between the blood volume values of infants from non-anemic and severely anemic mothers. The average blood volume of the latter group was 95.5 ml/kg. Mean values for the 12 infants of treated women was 100.8 ml/kg, and the mean of 13 infants of untreated women was 90.6 ml/kg.

The differences in blood volume between the groups cannot be accounted for solely by the differences in plasma volume: the disparity in red cell volumes also contributes.

(D.) *Total Red Cell Volume:* The mean red cell volume of the non-anemic infants* was 46.2 ml/kg, of the moderately anemic group

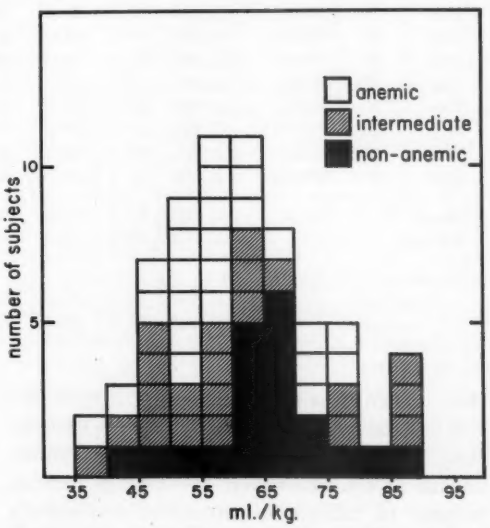


Fig. 1. The distribution of plasma volumes of individual subjects, and indicating the group in which each is placed.

*The terms "non-anemic" and "anemic" when applied to infants refer to their mothers' hematologic status.—*Editor's Note*

42.4 ml/kg, and of the anemic infants* 37.3 ml/kg. These differences reflect the values of the mothers in late pregnancy and to almost the same magnitude. The distribution of individual red cell volumes is shown in Figure 2.

There was no significant difference in mean red cell volumes of the non-anemic infants whether or not the mothers were treated with iron. There was a 2.4 ml/kg larger mean red cell volume in the infants of treated mothers than of untreated mothers in the severely anemic group. However, the average red cell volume of infants of untreated moderately anemic women was 4.1 ml/kg greater than that of infants of treated mothers in the same group. This unexpected discrepancy may be explained by the possibility that the intake or absorption of iron may have been inadequate in some women. At any rate, one need not necessarily conclude that iron therapy was ineffective in preventing this anemia of the newborn.

(E.) *Total Circulating Hemoglobin Mass:* The mean circulating hemoglobin mass of the non-anemic infants was 18.9 g/kg. The mean value of the moderately anemic group was 16.9 g/kg, nearly 10 per cent lower than the first group. The mean mass of the more severely anemic infants was 20 per cent lower than that of the non-anemic infants.

The average total circulating hemoglobin mass *not calculated on the basis of body weight* indicates the same large difference between the

TABLE III

Infants of Anemic Mothers

Name	Sex	Rx of mother	Birth weight g	Hgb g %	PRCV %	Plasma volume ml/kg	Blood volume ml/kg	Red cell volume ml/kg	Hb Mass g/kg	Hb Mass g	Serum Iron γ %	Serum Iron γ/kg
Bet	F	None	2,500	16.0	46.0	76.2	122.9	46.8	19.7	45.4	153	117
Bra	F	$FeSO_4$	3,630	17.1	48.0	72.1	119.6	47.4	20.4	74.2	103	74
All	M	$FeSO_4$	2,770	13.9	41.0	73.5	111.2	37.7	15.5	43.6	122	90
And	F	None	2,600	14.7	42.5	50.6	78.1	27.4	11.5	30.6	58	29
Bea	F	None	3,700	15.9	48.5	63.5	106.0	42.5	16.9	56.8	62	39
Bos	M	None	3,000	17.5	50.0	62.5	106.5	44.0	18.6	55.9	111	69
Bur	M	$FeSO_4$	3,350	15.1	48.0	56.7	93.9	37.3	14.2	47.2	59	34
Dic	F	$FeSO_4$	4,400	16.2	48.5	56.6	94.4	37.9	15.3	67.3	—	—
Fel	M	None	2,500	15.1	47.0	59.6	97.4	37.8	14.7	38.2	128	76
Hil	M	$FeSO_4$	3,650	12.3	34.0	61.1	84.9	23.9	10.4	35.7	77	47
Hun	F	None	2,760	17.0	51.5	45.6	79.3	33.7	13.5	38.6	52	24
Jen	M	None	2,960	16.0	49.0	47.9	80.5	32.6	12.9	38.1	81	39
Mar	M	None	3,110	17.7	56.0	55.8	102.3	46.5	18.1	54.5	89	50
Moy	F	None	3,000	16.7	54.0	56.6	102.2	45.6	17.1	51.2	—	—
Mul	F	$FeSO_4$	3,150	18.5	54.0	55.1	99.6	44.5	18.4	55.8	75	41
Per	M	$FeSO_4$	3,420	15.8	51.0	54.2	93.8	39.5	14.8	49.2	42	23
Pin	F	$FeSO_4$	3,230	16.2	48.0	65.4	108.5	43.0	17.6	53.6	149	97
Pin	F	None	2,590	15.0	43.0	42.5	65.9	23.4	9.9	26.1	75	32
Sha	F	$FeSO_4$	4,090	14.2	43.0	48.8	75.8	26.9	10.8	44.1	115	56
Smi	F	None	2,700	13.3	46.0	54.4	87.3	33.4	11.7	31.4	100	54
Syk	F	None	2,870	16.0	44.0	54.2	85.1	31.0	13.6	39.2	100	54
Tho	F	$FeSO_4$	3,500	17.3	51.5	73.4	127.8	54.4	22.1	73.4	56	41
Wil	M	$FeSO_4$	3,420	13.2	39.0	78.3	115.5	37.2	15.2	51.2	53	42
Wro	M	None	3,200	15.7	48.0	38.6	64.0	25.4	10.1	31.5	67	26
Hol	F	$FeSO_4$	3,400	16.7	48.0	50.7	84.1	33.4	14.1	47.1	116	59
Mean			3,180	15.7	47.2	58.2	95.5	37.3	15.1	47.2	89	53
Median			3,150	16.0	48.0	56.6	94.4	37.7	14.8	47.1	81	47
Means of:												
12 treated			3,501	15.5	46.2	62.2	100.8	38.6	15.7	53.5	88	53
13 untreated			2,884	15.9	48.1	54.5	90.6	36.2	14.5	41.3	90	51

TABLE IV

Mean Values of Mothers and Infants

No.		Hgb g %	PRCV %	Plasma volume ml/kg	Blood volume ml/kg	Red cell volume ml/kg	Hgb mass g/kg	Serum iron γ %	Serum iron γ/kg
	Mothers								
66	Non-anemic	11.2	34.7	78.1	111.4	33.6	12.5	80	62
	Intermediate	10.4	33.4	70.4	99.0	28.6	10.2	61	41
	Anemic	9.9	31.6	58.5	80.5	22.0	7.9	67	40
28	Treated (oral iron)								
	Non-anemic	10.9	34.4	78.8	111.6	33.4	12.1	84	67
	Intermediate	11.4	35.7	62.9	91.1	28.2	10.2	81	50
	Anemic	9.6	31.0	60.8	83.0	22.2	7.9	63	40
38	Untreated								
	Non-anemic	11.5	34.9	77.6	111.3	33.8	12.7	77	60
	Intermediate	9.8	31.9	75.1	103.9	28.8	10.1	49	36
	Anemic	10.2	32.1	57.4	78.1	21.8	7.9	70	39
	Infants								
66	Non-anemic	16.8	49.7	65.6	111.7	46.2	18.9	93	61
	Intermediate	16.5	50.0	60.8	103.2	42.4	16.9	84	53
	Anemic	15.7	47.2	58.2	95.5	37.3	15.1	89	53
28	Treated mothers								
	Non-anemic	17.1	50.6	64.2	110.9	46.7	19.2	117	77
	Intermediate	15.7	47.8	62.0	101.8	39.9	15.8	104	67
	Anemic	15.5	46.2	62.2	100.8	38.6	15.7	88	55
38	Untreated mothers								
	Non-anemic	16.6	49.1	66.5	112.3	45.8	18.7	79	51
	Intermediate	17.0	51.4	60.0	104.0	44.0	17.7	72	44
	Anemic	15.9	48.1	54.5	90.6	36.2	14.5	90	51

TABLE V

Serum Iron

Group	Combined average		Treated mothers		Untreated mothers	
	Concentration μg %	Total μg/kg	Concentration μg %	Total μg/kg	Concentration μg %	Total μg/kg
Non-anemic	93	61	117	77	79	51
Intermediate	84	53	104	67	72	44
Anemic	89	53	88	55	90	51

anemic and non-anemic groups. The average weight of the severely anemic infants was 3,180 g, and the average hemoglobin mass was 47.2 g. The non-anemic infants had a similar mean weight of 3,314 g, and a mean hemoglobin mass of 61.2 g. The difference of the means, 14.0 g of hemoglobin, represents 47.6 mg of iron.

The distribution of individual circulating hemoglobin mass values is illustrated in Figure 3.

(F.) *Serum Iron:* The mean serum iron concentration of the non-anemic infants was 93 μg/100 ml, that of the intermediate or moderately anemic group of infants was 84 μg/100 ml and that of the more severely anemic infants was 89 μg/100 ml. The differences are not significant since these average values include those of infants of both treated and untreated mothers, and since these figures represent concentrations in babies of various blood volumes.

A further delineation of these values may be gained by considering the total circulating serum-bound iron. The average circulating serum iron of the non-anemic infants was 61

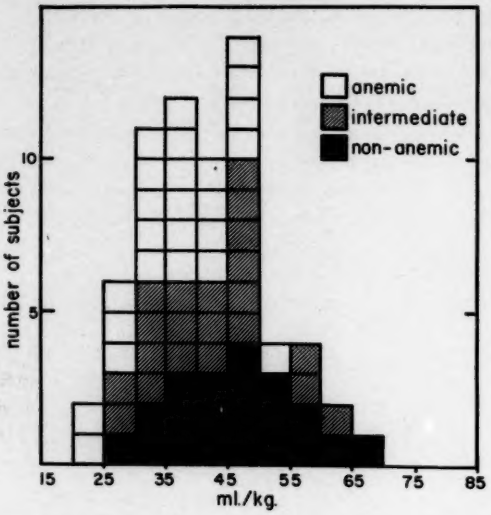


Fig. 2. The distribution of red cell volumes of individual subjects, and indicating the group in which each is placed.

μg/kg, and the mean was 53 μg/kg in both the moderately and severely anemic infants. The difference is of doubtful significance.

Subdivision of the three groups into those infants whose mothers received oral iron therapy and those whose mothers did not, presents an interesting pattern. Table V lists the values for both serum iron concentration and total serum iron per kilogram of body weight.

The combined mean serum iron concentrations exhibit no significant differences, though the combined total circulating values may. The infants of untreated severely anemic mothers had levels of serum iron higher than those of untreated moderately anemic mothers, and slightly higher than those of non-anemic mothers. This would indicate that the serum iron, no matter how measured, reflects not the serum iron of the mother, but the availability of this element for transplacental passage. It also indicates that measurement of maternal

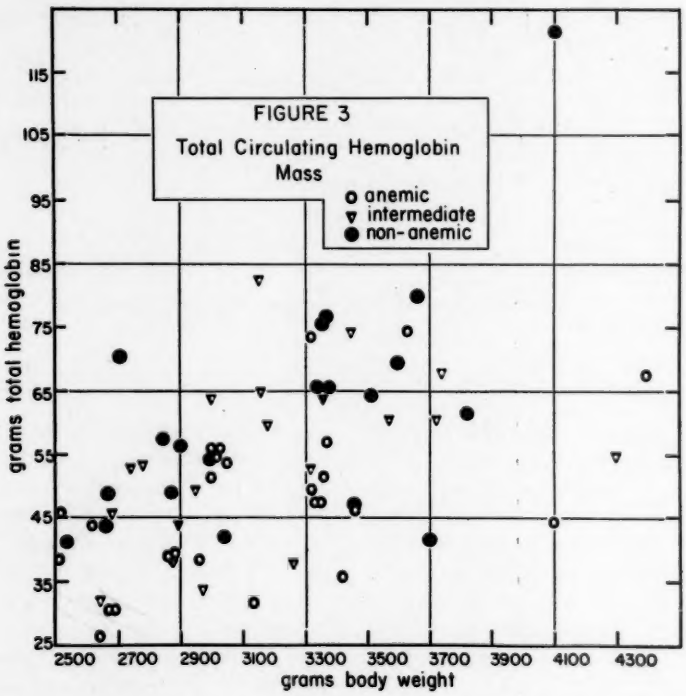


Fig. 3. Total circulating hemoglobin mass values of individual subjects, and indicating the group in which each is placed.

serum iron is a poor index of the relative state of anemia in the newborn.

(G.) *Serum Iron-binding Capacity:* The serum iron-binding capacity is usually totally saturated or nearly so in the newborn infant. Such was found to be true in the subjects of this investigation, therefore, the latent serum iron-binding capacities are not recorded.

DISCUSSION

In any study of the blood volume of the newborn it is necessary to consider the effect of the so-called "placental transfusion" which is said to occur as the result of delayed ligation of the umbilical cord.[18,19] Such a volume of blood, estimated by some observations to exceed 60 ml, would add substantially to the iron reserve of the newborn infant. It may also have had the effect of enlarging the blood volumes of some of the infants in this study.

We have determined the blood volumes of 38 infants with and without the influence of gravity on umbilical blood flow. Neither immediate nor delayed clamping of the cord with the infant held above or below the level of the placenta could be shown to affect significantly the total blood or red cell volumes measured by the fourth day of life. By "milking" the cords of one group of infants we were able to produce a plethora. We believe that neonatal volumes are generally unrelated to "placental transfusion" unless artificial maneuvering of the cord and placenta surround the technic.[20]

The fact that the lowest values for plasma and total blood volume were encountered in the anemic infants of untreated mothers might seem to indicate that iron deficiency leads to lower than normal blood volume figures. This may be true, but cannot be proved by the results of this investigation. Infants with hypovolemia and hypervolemia were found in all groups.

The outstanding influence of maternal iron deficiency was on the red cell volume and circulating hemoglobin mass. Mothers who had either presumably adequate iron stores or effective treatment with iron gave birth to infants with a superior content of red cells and hemoglobin. It was equally apparent that treatment of the severely anemic mothers, though not totally effective, assured the offspring of a better red cell volume and hemoglobin mass than infants of similarly deficient mothers without treatment.

The difference between the mean hemoglobin mass values of non-anemic and anemic infants was 3.8 g/kg. This is more striking when it is considered that this amount of hemoglobin represents 20 per cent of the iron otherwise available to the anemic infants for hemoglobin synthesis in the following months of life, unless supplied by diet or medicinal iron.

We feel that a serum iron value alone is not sufficiently indicative of iron deficiency in the newborn, since it relates to current iron turnover, and is in such a sensitively balanced state that many factors can influence the values found at any one time. Determination of the serum iron concentration and latent iron-binding capacity in later infancy, childhood, and adulthood are often of assistance in the diagnosis of iron deficiency anemia, but not in the newborn period. At that time iron stores reside principally in the hemoglobin iron, and it is thought that other depots of iron are inconsequential. Thus it appears that the serum iron of the newborn infant reflects the maternal iron supply, but is not directly related to the maternal serum iron levels. One can infer that the simple process of administering iron to the mother does not always ensure an adequate passage of iron across the placenta. The demands for iron by the fetus are not entirely met at the mother's expense if she is anemic, for she will satisfy, at least in part, her own needs. Treatment of the mother with oral iron during the last trimester or longer seems to produce a more abundant circulation of iron in the newborn infant regardless of her relative hematologic status. Babies of treated mothers had, in general, higher total circulating serum iron levels than those of untreated mothers. Among the individuals of the anemic group no greater benefit was exerted by maternal therapy than by natural maternal iron stores and the provisions of diet.

We have seen that it is rare for a non-anemic mother to give birth to an infant with evidence of iron deficiency anemia; but our data strongly suggest that infants of anemic mothers

will frequently share the iron deficiency.

It is concluded that the more severe the anemia in the mother (see Table IV) the more this will be reflected in the infant at birth. Such a profound effect on the newborn, depriving it of nearly one quarter of its readily available iron store, may be expected to influence the production of iron deficiency anemia in later months of life.

SUMMARY

Sixty-six pregnant women (25 of them severly anemic, 21 moderately or mildly anemic, and 20 not anemic), and later their newborn infants, were studied by standard hematologic methods and by volumetric technics. The diagnosis of maternal iron deficiency anemia was made from values established in the laft half of the third trimester of pregnancy.

The mean red cell volume of infants of non-anemic mothers was 46.2 ml/kg; that of the infants of severely anemic mothers was 37.3 ml/kg, a value 19 per cent below that of the normal group.

The mean circulating hemoglobin mass of the non-anemic group was 18.9 g/kg; that of the anemic group 15.1 g/kg. The difference of 3.8 g/kg represents a deficiency of about 20 per cent of the iron otherwise available to the anemic infants compared with the normal newborns.

Infants of mothers treated with oral iron during pregnancy had, in general, higher total circulating serum iron levels than infants of untreated mothers.

It is concluded that infants of anemic mothers will frequently share the iron deficiency, and that treatment of the mother with iron during her pregnancy tends to relieve her infant of this common deficiency. The more severe the anemia in the mother the more this will be reflected in the infant at birth. Such a profound effect on the newborn may be expected to influence the production of iron deficiency anemia in later infancy.

ACKNOWLEDGMENT

The authors gratefully acknowledge the expert technical assistance of Miss Lorraine Whalen and Miss Amalia Telek throughout the course of this investigation.

REFERENCES

1. Strauss, M. B.: Anemia of infancy from maternal iron deficiency in pregnancy. *J. Clin. Investigation* 12: 345, 1933.
2. Woodruff, C. W. and Bridgeforth, E. B.: Relationship between the hemogram of the infant and that of the mother during pregnancy. *Pediatrics* 12: 681, 1953.
3. Oettinger, L., Jr., Mills, W. B., and Hahn, P. F.: Iron absorption in premature and full-term infants. *J. Pediat.* 45: 302, 1954.
4. Woodruff, C. W.: Maternal factors in hypochromic anemia of infancy. *J.A.M.A.* 162: 659, 1956.
5. Woodruff, C. W.: The multiple causes of hypochromic anemia. (In press.)
6. Guest, G. W. and Brown, E. W.: Erythrocytes and hemoglobin of the blood in infancy and childhood, III. Factors in variability. *A.M.A. J. Dis. Child.* 93: 486, 1957.
7. Lund, C. J. and Sisson, T. R. C.: Volumetric studies in pregnancy. (In preparation.)
8. Crosby, W., Munn, J. L., and Furth, F.: Standardizing a method for clinical hemoglobinometry. *U. S. Armed Forces M. J.* 5: 693, 1954.
9. Nitsche, G. A. and Cohen, P. P.: Simplified determination of blood volume. *Am. J. Clin. Path.* 17: 239, 1947.
10. Allen, T. H.: Extraction of T-1824 in the presence of gross hemolysis and lipemia. *Proc. Soc. Exper. Biol. & Med.* 76: 145, 1951.
11. Chaplin, H. and Mollison, P. L.: Correction for plasma trapped in the red cell column of the hematocrit tube. *Blood* 7: 1227, 1952.
12. McGovern, J. J., Jones, A. R., and Steinberg, A.G.: The hematocrit of capillary blood. *New England J. Med.* 253: 308, 1955.
13. Mollison, P. L.: *Blood Transfusion in Clinical Medicine*, ed. 2. Thomas, Springfield, Ill., 1956, p. 62.
14. Burch, H. B., Lowry, O. H., and Bessey, O. A.: The determination of iron in small amounts of blood serum. *J. Biol. Chem.* 174: 791, 1948.
15. Rath, C. E. and Finch, C. A.: Chemical, clinical, and immunological studies on the products of human plasma fractionation, XXXVIII. Serum iron transport, measurement of iron-binding capacity of serum in man. *J. Clin. Investigation* 28: 79, 1949.
16. Sisson, T. R. C. and Lund, C. J.: The blood volume in infants, I. The full-term infant during the first year of life. (In preparation.)
17. Sisson, T. R. C. and Lund, C. J.: The blood volume in infants, II. The premature infant during the first year of life. (In preparation.)
18. DeMarsh, Q. B., Windle, W. F., and Alt, H. L.: Blood volume of the newborn infant in relation to early and late clamping of the umbilical cord. *A.M.A. J. Dis. Child.* 63: 1123, 1942.

19. DUCKMAN, S., MERK, H., LEHMAN, W. X., and REGAN, E.: The importance of gravity in delayed ligation of the umbilical cord. *Am. J. Obst. & Gynec.* 66: 1214, 1953.

20. WHIPPLE, G. A., SISSON, T. R. C., and LUND, C. J.: Delayed ligation of the umbilical cord, its influence on the blood volume of the newborn. *Obst. & Gynec.* 10: 603, 1957.

Beyond the Facts

"It is not easy for the student or physician to move through a busy career and maintain a lively curiosity. Too often we encounter satisfaction and even complacency in the statement, we must not go beyond the facts. I belong to a school which holds that we must encourage our pupils to let fancy roam where it will. The danger-line between fact and fancy will never be obscured when fancy is habitually encouraged to formulate tests whereby its truth may be examined."
—A. Weech, *J. Med. Education* 31: 106, 1956

Factors Affecting the Absorption of Vitamin B_{12}

BACON F. CHOW, PH.D., JENG M. HSU, PH.D., KUNIO OKUDA, M.D., RALPH GRASBECK, M.D.,* AND ANDREW HORONICK, B.S.

PREVIOUS publications from this laboratory and others have described the variations in serum vitamin B_{12} levels, and in the absorption of vitamin B_{12} that are associated with advancing age,[1] pregnancy,[2] hypothyroidism,[3] ACTH and cortisone administration or excess secretion,[4] pyridoxine deficiency,[5] the administration of various intrinsic factor preparations,[6] and the administration of a multiple vitamin-lipotropic factor elixir containing sorbitol in the vehicle.[7] The general findings are summarized in Table I.

The purpose of this paper is to present our additional findings on the effects on the blood levels and absorption of vitamin B_{12} of (1) gastrectomy, (2) divided dose schedules, and (3) the physical state of the orally administered vitamin. We also present evidence further substantiating the effects of pyridoxine deficiency upon vitamin B_{12} absorption and tissue content.

METHODS FOR MEASURING VITAMIN B_{12} ABSORPTION

The absorption of orally administered radioactive vitamin B_{12} tagged with Co^{60} can be measured by the urinary[8] or fecal excretion[9] tests or by hepatic uptake test.[10] Absorption may be estimated from radiometric measurements of feces, scintillation counting of liver projections, or determination of urinary radioactivity estimated after injection of a massive dose of non-radioactive vitamin B_{12}.

In view of a general and understandable hesitancy on the part of investigators to allow the test subjects, particularly infants or pregnant women, to be exposed to the hazards of radioactivity, however safe this may be, the oral tolerance test[11] for estimating absorption is often preferred under such conditions. This test involves either the oral administration of a single dose of 1,000 μg or a daily dose of physiologic magnitude. Increase in serum vitamin B_{12} levels is estimated by a microbiologic assay and is taken as an index of absorption. When

TABLE I*

Variations in Serum Vitamin B_{12} Levels and in Absorption of Vitamin B_{12}

Condition or treatment	Effect on blood level of B_{12}	Effect on absorption of B_{12}
Aged	Decreased	Normal†
Pregnancy	Decreased	Increased
Hypothyroidism	Decreased	Decreased
ACTH or cortisone	Increased	Normal
Pyridoxine deficiency	—	Decreased
"Inhibitory" I. F.	Decreased	Decreased
"Non-inhibitory" I. F.	Increased	Increased
Liptril®	Increased	Increased

* These findings apply to subjects with gastric secretions containing endogenous intrinsic factor.

† Although elderly subjects were found to absorb vitamin B_{12} administered in the fasting state as well as young healthy adults, it was found that young adults responded to an injection of histamine with an increased absorption of vitamin B_{12}, whereas the elderly did not.[7a]

From the Department of Biochemistry, School of Hygiene, Johns Hopkins University, Baltimore, Maryland, and Institute of Geriatrics, Bronx, New York.

* Present address: Fourth Medical Clinic, University of Helsingfors, Helsingfors, Finland.

Presented at the *Symposium on Some Recent Observations on Biological Relationships* held in New York City, March 5, 1957, with the cooperation of The National Vitamin Foundation.

The authors wish to acknowledge with thanks the Grants-in-Aid or supply of materials from The National Vitamin Foundation, Atomic Energy Commission AT (30-1) 1203, U.S.P.H.S. (C3), Merck and Company, and Lederle Laboratories.

a single large dose is given, the test involves the drawing of blood one to three hours after the administration. When a small daily dose is used, blood specimens are collected over a longer period of time (in weeks or months) depending on the efficacy of medication. The vitamin B_{12} content in sera is then determined and compared with that found before treatment. Although the oral tolerance test simulates the actual conditions of use, the application of the radiometric measurement has numerous advantages.

Since the details of the above mentioned methods of measurements have been published elsewhere, they will not be repeated in this communication.

RESULTS

Absorption of Vitamin B_{12} by Totally Gastrectomized Subjects: In these patients, the stomach had been removed in its entirety as attested to not only by the surgeons' notes, but also by the pathologist as well as by subsequent radiologic and endoscopic examinations. In some, the stomach had been removed because of malignancy and, in others, benign ulceration. All the subjects studied were between 50 and 70 years of age. Two of the subjects were given test doses of 3 mg, whereas the other two were given 1 mg. Sera samples were obtained shortly before, one and one-half and three hours after administration, and were then analyzed for vitamin B_{12} activity. It can be seen (Table II) that subjects with total gastrectomy showed no measurable vitamin B_{12} activity in the initial sera. However, the subsequent specimens contained high concentrations of vitamin B_{12}. (The average value for our group of healthy individuals is 210 ± 40 $\mu\mu$g/ml.) Therefore, absorption of the vitamin must have taken place.

TABLE II

Oral Tolerance Test of Vitamin B_{12}

Subject*	Years after gastrectomy	B_{12} given *mg*	Vitamin B_{12} serum levels ($\mu\mu$g/ml)		
			0 *hr*	1.5 *hr*	3 *hr*
J. W. (a)	8	3	0	198	262
E. W.	7	3	0	1280	1500
J. W. (b)	6	1	0	186	192
J. P.	9	1	0	810	700

* All subjects underwent total gastrectomy.

In the second experiment, another series of five subjects with total gastrectomy was used. The test dose of vitamin B_{12} was 1 mg in all instances. It can be seen again (Table III) that the administration of this dose of the vitamin by mouth brought about a definite increase within three hours, the exception being H. D. who did not show the standard response (an increase of 150 $\mu\mu$g/ml). Upon re-test, two weeks later, it was found that H. D. gave a very marked response with an increase of 350 $\mu\mu$g/ml in two hours. These results again demonstrate the low initial vitamin B_{12} serum level and the ability of totally gastrectomized subjects to respond to a 1,000 μg test dose of vitamin B_{12} given by mouth. It should be noted that the vitamin B_{12} serum level of the gastrectomized subjects returned to the original low values in about two weeks.

The absorption of vitamin B_{12} by gastrectomized subjects was determined with the urinary excretion test at two levels of oral intake, namely, 2 and 1,000 μg. Thus, subjects H. D. and W. H. C. were given 2 μg of cobalt^{60}-labeled vitamin B_{12} (specific activity = 180 μc/mg) by mouth followed by the injection of 1 mg of unlabeled vitamin B_{12} two hours later. It was found (Table IV) that the total amounts of radioactivity which appeared in the 24-hour urine specimen were 0.18 and 2.8 per cent, respectively; whereas, our experience[12] shows that healthy subjects with intact stomachs would excrete about 10 per cent of this orally administered dose. Thus, the gastrectomized subjects, like those with pernicious anemia, absorbed a small amount of the orally fed vitamin B_{12}. However, when 1,000 μg of this vitamin, prepared by mixing 2 μg of radioactive vitamin B_{12} with 998 μg of unlabeled vitamin B_{12}, was fed to four totally gastrectomized subjects (two of them received 2 μg radioactive vitamin B_{12} in the previous test), it can be seen that at the higher dose, as much as 36 μg (M. L.) of vitamin B_{12} representing 3.6 per cent of the administered dose, appeared in the urine. This amount represents only a small

TABLE III

Oral Tolerance Test for Vitamin B_{12} Absorption by Gastrectomized Subjects

Subjects	Dosage given *mg*	Vitamin B_{12} serum levels ($\mu\mu g/ml$)					
		0 *hr*	1.5 *hr*	3 *hr*	8 *days*	16 *days*	28 *days*
E. W.	1.0	35	1,450*	1,750*	193	97	47
H. D.	1.0	41*	52	122	41	29	—
J. P.	1.0	35	550*	580*	82	55	23
J. W. (a)	1.0	<50	198	262	70	58*	58*
W. J. C.	1.0	41	286	373	111	60	53

* = approximately.

fraction of the absorbed vitamin B_{12} and is considerably larger than that excreted by non-gastrectomized subjects under similar test conditions. These data, therefore, indicate that the rise in the microbial activity in serum following oral administration of a large dose is due to the increased absorption of the orally administered vitamin.

TABLE IV

Urinary Excretion Test of Radioactive Vitamin B_{12} Absorption by Gastrectomized and Non-gastrectomized Subjects

Subject (gastrectomized)	Administered radioactive vitamin B_{12}	Radioactivity in 24 hr urine	
	μg	$m\mu g$	%
H. D.	2	3.6	0.18
W. H. C.	2	55	2.8
H. D.	1,000	22,000	2.2
E. W.	1,000	12,000	1.2
M. L.	1,000	36,000	3.6
W. H. C.	1,000	27,000	2.7
(Non-gastrectomized)			
5 subjects	2	220 ± 15	11.0
5 subjects	1,000	3,600 ± 580	0.36

The Importance of Physical State and Chemical Substances with which Vitamin B_{12} Is Incorporated: (1) Effect of administration in divided dosages on urinary excretion. The cobalt^{60}-labeled vitamin B_{12} in various amounts (2.0 μg, 8.0 μg, and 50 μg)* was given by mouth to two groups of clinically healthy subjects. One group received one of the above mentioned quantities in one single dose in 20 ml of water with additional 50 ml water in five portions for rinsing. The second group received the same amounts of radioactive vitamin B_{12} in four divided doses at intervals of 15 minutes. The total water intake, including that used for rinsing purposes was the same for both groups. Results tabulated in Table V demonstrate that the administration of a total amount of 2 μg in divided doses resulted in a slight increase in the radioactivity in the 24-hour urine over the group receiving the same amount of radioactive vitamin B_{12} in single doses. The difference,

* The total radioactivity taken by each individual was 0.36 μc, regardless of the total dosage of vitamin B_{12}.

TABLE V

Effect of Administration in Divided Dosages on Urinary Excretion of Radioactive Vitamin B_{12}

Expt.	Total *mcg* administered	Doses	Number of subjects	$m\mu g$ of B_{12} in 24-hour urine*	P* value*
A	2.0	4	5	256 ± 30	>0.05
	2.0	1	5	220 ± 16	
B	2.0	4	8	244 ± 38.1	>0.05
	2.0	1	8	210 ± 21.3	
A	8.0	4	5	500 ± 51.2	<0.05
	8.0	1	5	335 ± 38.7	
B	8.0	4	10	486 ± 39.2	<0.01
	8.0	1	10	330 ± 18.1	
A	50.0	4	5	630 ± 42.1	<0.05
	50.0	1	5	502 ± 38.0	
B	50.0	4	8	721 ± 66.4	>0.05
	50.0	1	8	561 ± 60.2	
C	50.0	4	7	574 ± 42.2	<0.05
	50.0	1	7	454 ± 34.8	
D	50.0	4	7	602 ± 39.8	<0.02
	50.0	1	5	442 ± 28.2	

* Probabilities of differences in the means as determined by the Fisher test.

however, is not statistically significant. When the doses were increased to 8 or 50 μg, a significant increase in the urinary excretion was observed in six separate experiments involving the use of 70 healthy individuals, in favor of the divided doses. It was thought to be plausible that the effect of the divided doses of 8 μg or more may be due to the insufficiency of intrinsic factor needed for the absorption of this large amount of vitamin B_{12}. When a non-inhibitory intrinsic factor concentrate was used, actual enhancement in the urinary excretion was observed. Thus, one group of twelve subjects receiving 50 μg of radioactive vitamin B_{12} alone excreted on the average 512 ± 37 mμg of radioactive vitamin B_{12} in 24 hours, whereas another group of 12 subjects given 50 mμg of radioactive vitamin B_{12} plus four daily oral doses of an intrinsic factor concentrate (Neofactrin®)* gave a mean value of 628 ± 41 μg; the difference is statistically significant. In a like manner, it was found that when 25 μg of vitamin B_{12} were co-administered daily with non-inhibitory intrinsic factor† to 15 pregnant women from their third trimester to the time of delivery, the average vitamin B_{12} serum level was increased from 150 ± 21 μμg/ml to 185 ± 15 μμg/ml. However, when 25 μg of vitamin B_{12} alone was administered to another 15 pregnant women, the vitamin B_{12} serum level dropped from 168 ± 15 μμg/ml to 111 ± 12 μμg/ml.

(2) The physical state of vitamin B_{12} administered: Inasmuch as the site and the mechanism of absorption of vitamin B_{12} are poorly understood, we wished to ascertain whether the physical state in which the vitamin B_{12} is to be administered may play an important role in absorption. In experiment I, two groups of subjects were administered 2 μg of radioactive vitamin B_{12} in hard gelatin capsules (a) containing other vitamins, or in solution (b), respectively. Two μg of radioactive vitamin B_{12} was injected quantitatively with a syringe into the capsule, which was subsequently sealed with molten gelatin. The fluid intake, including that for rinsing, of both groups of subjects at the time of testing, was limited to 60 cc of water. Two hours after the administration of the radiovitamin, each subject received intramuscularly 1,000 μg of the unlabeled vitamin. The total radioactivity in the 24-hour urine specimen was measured by scintillation counting. It can be seen (Table VI) that two out of six subjects (group A, experiment I) receiving capsules excreted unusually small amounts of radioactivity in the urine, whereas those receiving the same amount of the radiovitamin in solution excreted uniformly pure. Three months afterwards, the same subjects were again used for testing, except that those who had previously received vitamin B_{12} in solution, now received it in capsules. A third group (C) of individuals was also used. They received, in solution, the same vitamins that group A received in the capsules, in order

TABLE VI

Effect of Capsule on Urinary Excretion of Radioactive Vitamin B_{12} in Six Subjects

	Experiment I		Experiment II		
	A*	B†	A*	B†	C‡
	226	240	180	276	194
	50	210	30	198	210
	40	170	200	208	246
	200	190	240	230	310
	176	220	36	176	188
	152	230	76	290	146
Mean	140.6 ± 31.9	210 ± 10.7	127 ± 37.1	229 ± 18.4	215.7 ± 23.1

All figures are mμg of radioactive vitamin B_{12} in the 24-hour urine.

Subjects used in group A (experiment I) were same as those in B (experiment II).

Subjects used in group B (experiment I) were same as those in A (experiment II).

Subjects used in group C (experiment II) were different subjects.

* A = capsule (Gevral + 2 mcg vitamin B_{12}) vitamin B_{12} was injected and sealed.

† B = 2 mcg vitamin B_{12}*.

‡ C = 2 mcg vitamin B_{12}* + content of Gevral® in suspension.

* Neofactrin was kindly supplied by the Stuart Company.

† We wish to thank Stuart Company for their supply of Prenatal capsules. The intrinsic factor preparation used contained intrinsic factor activity according to the standard U.S.P. test and would also aid absorption of orally administered vitamin B_{12} by clinically healthy subjects according to the urinary excretion test.

to be certain that the observed differences were not due to any reaction between vitamin B_{12} and some chemical substances. Three subjects who showed normal excretion patterns upon the receipt of vitamin B_{12} in solution now excreted small amounts of radioactivity in the urine. The results demonstrate that the vitamin B_{12} in these specific capsules was not absorbed uniformly well by the test subjects, possibly because the capsules did not dissolve with sufficient rapidity in some subjects.

TABLE VII

Composition of "Elixir"

Ingredients	Per 5 ml
Vitamin B_{12} (crystalline)	8.34 μg
Riboflavin	0.6 mg
Niacinamide	7.0 mg
Pyridoxine	2.0 mg
Betaine (anhydrous)	700.0 mg
Choline dihydrogen citrate	150.0 mg
Inositol	150.0 mg
Ferric pyrophosphate	35.0 mg
Caffeine citrate	65.0 mg
Alcohol (Sorbitol used as vehicle)	15 %

These findings on the relatively poor absorption of vitamin B_{12} provided in these specific capsules received additional experimental confirmation from another study with three groups of elderly subjects (clinically healthy and ambulatory residents of the Institute of Geriatrics in New York). Three groups of 12 subjects each received daily the following treatments:

Group A—100 μg of vitamin B_{12} in a hard gelatin capsule containing other vitamins.

Group B—100 μg of vitamin B_{12} in an aqueous solution together with a vitamin capsule of the same composition of that used in Group A, except vitamin B_{22} was absent.

Group C—25 μg of vitamin B_{12} in a lipotropic elixir[7] (Smith, Kline and French Laboratories*).

Serum specimens were obtained from the subjects in all three groups at regular intervals for the determination of the vitamin B_{12} activity. The results of this study are tabulated in Table VIII. The initial serum vitamin B_{12} levels in all three groups were low and statistically indistinguishable. One month after treatment, the serum vitamin B_{12} level of those receiving the elixir was elevated significantly. After four months, there was only a slight increase in group A, but marked increases in groups B and C. The elevation was more pronounced in Group C than in group B. Six months afterwards, the level of group A was essentially the same as that of group C after only one month of administration at one-quarter of the daily dose. Treatment with 100 μg of vitamin B_{12} in solution for six months resulted in an elevation equal to that of 25 μg of vitamin B_{12} in an elixir administered for four months.

Effect of Pyridoxine Deficiency: The effect of pyridoxine deficiency[5] on the absorption of vitamin B_{12} was studied with adult male and female rats. After ten weeks of feeding a pyridoxine-deficient diet, the male animals lost 18 g each, whereas those treated with pyridoxine gained 54 g each; thus, the algebraic difference between the changes in mean body weights of these two groups was 74 g. The mean body weight of the treated female controls remained unchanged after ten weeks, but was 33 g higher than those of pyridoxine-de-

TABLE VIII

Physical State of Administration of Vitamin B_{12}

Group	Vitamin B_{12}/day μg	Form of administration	Serum vitamin B_{12} levels in μμg months after treatment μμg/ml			
			0	1	4	6
A	100	Capsule	116 ± 21	—	192 ± 29	230 ± 36
B	100	Aqueous solution	112 ± 19	—	456 ± 38	662 ± 41
C	25	SKF elixir	120 ± 17	226 ± 15	675 ± 51	—

* See Table VII for composition of elixir.

ficient female rats. The results tabulated in Table IX demonstrate that the radioactivity in the fecal matter of the pyridoxine-treated male and female rats is consistently and significantly lower than those of the deficient rats, while the urinary excretion of the treated animals is higher than that of the deficient animals. The radioactivity in the target organs, such as liver and kidneys, is higher in the treated animals. It is of interest to note that radioactive vitamin B_{12} present in the gastrointestinal tract is highest among the deficient animals. These data taken as a whole suggest an impairment of vitamin B_{12} absorption related to pyridoxine deficiency.

If the impairment of vitamin B_{12} absorption elucidated above were due to pyridoxine deficiency, it may be expected that repletion with this vitamin will correct this defect unless the damage is irreversible. To this end, 12 young rats (group A) were placed on pyridoxine-deficient diets for a period of five weeks. A like number of animals (group B) were offered the same diet, but were treated with pyridoxine by injection. Five weeks later, six rats from group A, and an equal number from group B were randomly selected and given the oral test for vitamin B_{12} absorption with the procedure previously described. At the same time, the injection of pyridoxine to the remaining six rats in group A was started and was withdrawn from group B. This treatment was continued for eight weeks at which time the vitamin B_{12} absorption test was again applied. Our results (Table X) support our conclusion that pyridoxine deficiency impaired the absorption of vitamin B_{12}, and this can be fully corrected by treatment with pyridoxine.

TABLE IX

Effect of Pyridoxine Deficiency on Absorption of Vitamin B_{12}

Treatment	Radioactivity (per cent of oral dose)				
	Feces	Urine	Liver	Kidney	G.I. Tract
Adult male rats					
Pyridoxine deficiency	49.4 ± 3.4*	2.03 ± 0.24	6.2 ± 0.71	7.4 ± 0.40	10.8 ± 0.78
Pyridoxine treated	36.4 ± 4.2	3.25 ± 0.49	8.0 ± 0.60	8.8 ± 0.17	8.6 ± 0.65
Adult female rats					
Pyridoxine deficiency	59.8 ± 3.2	1.22 ± 0.12	6.2 ± 0.31	5.9 ± 0.49	10.1 ± 1.20
Pyridoxine treated	42.3 ± 2.7	1.92 ± 0.45	9.2 ± 0.45	10.5 ± 0.05	9.1 ± 1.01

* Standard error of the mean.

DISCUSSION

In spite of the availability of radioactive vitamin B_{12} little progress has been made in understanding the sites where the absorption of vitamin B_{12} can take place. While various methods have been proposed to estimate the absorption of vitamin B_{12}, each method has its own innate shortcomings which requires cautious interpretation of the results. For example, the interpretation of data obtained from the commonly used Schilling test,[8] as a measurement of vitamin B_{12} absorption, assumes equal retention of absorbed vitamin B_{12} by tissues of test subjects. Since the amount of vitamin B_{12} retained by the tissues is much greater than that excreted in the urine even after "flushing" by massive doses of unlabeled vitamin B_{12}, any small differences in tissue retention of vitamin B_{12} among different subjects may magnify the amount of urinary excretion. The fecal excretion procedure may appear to provide a direct measurement[9] of absorption of vitamin B_{12}. However, it ignores the possibility that absorbed vitamin B_{12} may be excreted through the bile and finally in the feces. This pathway of vitamin B_{12} elimination was demonstrated by Okuda *et al.*[13] The oral tolerance test is time-consuming and useful only for semiquantitative comparison. However, it need not involve the use of radioactive vitamins. Therefore, to understand the mechanism of absorption of vitamin B_{12} and the functions of various organs in the gastrointes-

tinal tract, the use of various types of patients may yield more informative data. For example, it was shown that feeding of radioactive vitamin B_{12} to totally gastrectomized subjects in small doses resulted in impaired absorption. On the other hand, if this vitamin is fed in large doses, the amounts of vitamin B_{12} appearing in serum or urine of gastrectomized subjects are higher than in normal subjects. From such data one may conclude that the absorption of vitamin B_{12} can take place in the absence of stomach, depending on the dose administered. Since absorption of vitamin B_{12} can take place, it is possible that the absorption of this vitamin could be affected by the divided dosage schedule and by the physical states in which vitamin B_{12} is administered. This belief is substantiated by the results of our studies in which it is shown that the amount of vitamin B_{12} absorbed is dependent on the physical state administered. Thus, vitamin B_{12} contained in at least one type of hard gelatin capsule is not uniformly absorbable; vitamin B_{12} in aqueous solutions is absorbed more easily.

TABLE X

Effect of Pyridoxine Repletion on Absorption of Vitamin B_{12} of Pyridoxine Deficient Female Rats

Group	Treatment	Average body weight			Radioactivity in per cent of administered dose			
		Initial g	End of 5 weeks g	End of 13 weeks g	Feces	Urine	Liver	Kidneys
A	Pyridoxine deficient	76 ± 2.1* (12)	120 ± 3.4	—	56.3 ± 3.4 (6)	1.22 ± 0.51	6.5 ± 0.54	6.3 ± 0.15
B	Pyridoxine treated	78 ± 1.4 (12)	164 ± 4.5	—	43.8 ± 4.1 (6)	1.85 ± 0.41	9.8 ± 0.61	8.4 ± 0.31
A	First 5 weeks (pyridoxine deficient) Second 8 weeks (pyridoxine injection)	—	—	204 ± 1.2 (6)	45.4 ± 3.1 (6)	1.91 ± 0.35	10.2 ± 0.41	8.5 ± 0.1
B	First 5 weeks (pyridoxine injection) Second 8 weeks (pyridoxine deficient)	—	—	173 ± 0.3 (6)	57.3 ± 4.5 (6)	1.16 ± 0.56	6.1 ± 0.39	6.1 ± 0

* Standard error of the mean.

Parentheses around numbers indicate number of rats used.

SUMMARY AND CONCLUSIONS

Data have been presented to show that absorption of vitamin B_{12} can take place in subjects without a stomach. Therefore, it must occur in the intestines or sublingually.

Absorption can be increased by the divided dosage schedule.

The absorption of vitamin B_{12} in clinically healthy subjects is partially dependent on the physical state in which it is administered. Vitamin B_{12} given in aqueous solutions to normal subjects is better absorbed than that given in a specific type of capsule.

Vitamin B_{12} absorption is also impaired by vitamin B_6 deficiency, and can be improved by subsequent administration of pyridoxine.

REFERENCES

1. Gaffney, G. W., Horonick, A., Okuda, K., Meier, P., Chow, B. F., and Shock, N. W.: Vitamin B_{12} serum concentrations in 528 "healthy" human subjects of ages 12–94. *J. Gerontology* 12: 1, 1957.
2. Hellegers, A., Okuda, K., Nesbitt, R. E. L., Jr., Smith, D. W., and Chow, B. F.: Vitamin B_{12} absorption in pregnancy and in the newborn. Am. J. Clin. Nutrition 5: 327, 1957.
3. Okuda, K., Steelman, S., and Chow, B. F.: Absorption of vitamin B_{12} in hyper- and hypothyroid rats. *Fed. Proc.* 15: 567, 1956.
4. Chow, B. F. and Stone, H.: The relationship of vitamin B_{12} to carbohydrate metabolism and diabetes mellitus. Am. J. Clin. Nutrition 5: 431, 1957.

5. Hsu, J. M. and Chow, B. F.: Effect of pyridoxine deficiency on the absorption of vitamin B_{12}. *Arch. Biochem.* 72: 2, 322, 1957.

6. Chow, B. F., Williams, W. L., Okuda, K., and Grasbeck, R.: Effect of crude and purified intrinsic factor preparation. Am. J. Clin. Nutrition 4: 142, 1956.

7a. Chow, B. F., Horonick, A., and Okuda, K.: Effect of an elixir on the absorption of vitamin B_{12} by healthy young and old subjects. Am. J. Clin. Nutrition 4: 434, 1956.

b. Tauber, S. A., Goodhardt, R. S., Hsu, J. M., Blumberg, N., Kassab, J., and Chow, B. F.: Vitamin B_{12} deficiency in the aged. *Geriatrics* 12: 368, 1957.

8. Schilling, R. F.: Intrinsic factor studies. II. The effect of gastric juice on the urinary excretion of radioactivity. *J. Lab. & Clin. Med.* 42: 860, 1953.

9. Heinle, R. W., Welch, A. D., Scharf, V., Meacham, G. C., and Prusoff, W. H.: Concentration of intrinsic factor and vitamin B_{12}-binding activities of fractions of desiccated hog stomach. *Blood* 8: 491, 1953.

10. Glass, G. J. B., Boyd, L. J., Gellin, G. A., and Stephenson, L.: Uptake of radioactive vitamin B_{12} by the liver in humans. Test for measurement of intestinal absorption of vitamin B_{12} and intrinsic factor activity. *Fed. Proc.* 13: 54, 1954; *Arch. Biochem.* 51: 251, 1954.

11. Chow, B. F.: Vitamin B_{12} and aging. Symposium on problems of gerontology—Johns Hopkins University, School of Hygiene & Pub. Health, and The National Vit. Foundation, Inc., New York. *Nutrition Series* 9: 59, 1954.

12. Chow, B. F., Gilbert, J. P., Okuda, K., and Rosenbloom, C.: Reproducibility of results and agewise variation. Am. J. Clin. Nutrition 4: 142, 1956.

13. Okuda, K., Grasbeck, R., and Chow, B. F.: Bile and B_{12} absorption. *J. Lab. & Clin. Med.* 51: 17, 1958.

Problems Connected with the Possible Use of Plankton for Human Nutrition

ERNEST GEIGER, M.D., PH.D.*

EVER SINCE it was recognized that proper nutrition of the world population represents a global problem of the highest importance, economists and nutritionists have been steadily searching for new sources of food supply. Besides the terrestrial sources which, according to some predictions, may not be able to keep up with the growth of the population, the ocean has been considered as the most promising source of nutrients. Today only a small fraction of the food grown in the ocean is utilized for human nutrition in the form of some surface fishes and other "sea foods."[34] Besides these, however, two further and possibly much richer sources are not yet even tapped. One of these is the so-called deep sea animal which lives in the bathypelagic zone and the other food source is represented by the drifting minute organisms known as zooplankton.

According to some authors the idea of utilizing the "pastures" of the ocean for food production is an ancient one, and Melville quoted in *Moby Dick*, as a proof, the following passage from Obed Macy's *History of Nantucket*, "In the year 1690 some persons were on a hill observing the whales spouting and sporting with each other when one observed: 'There'—pointing to the ocean—'is a green pasture where our children's grandchildren will go for bread.' "

Such predictions were however probably nothing better than poetic visions because the existence of plankton was discovered, according to some authors, only in 1828 by the British Army Surgeon Vaughn Thomas. Officially, however, the great physiologist Johannes Müller is credited with the first description of planktonic life in 1847.[17] He used a tow net for the first time for collecting plankton and reported the amazing richness of minuscule animals present in the ocean water around Helgoland. In 1872 the famous Challenger expedition supplied further important data to the morphology and ecology of plankton. The first official step toward the practical use of plankton for human nutrition seems to be the request of Sir John Graham Kerr in 1941 when he asked the British Parliament to investigate the harvesting and use of plankton as a possible means of relieving the food shortage resulting from the German submarine warfare. During the Second World War experiments were also initiated and supported by the United States Government, investigating the possible use of plankton as emergency food for survival on life rafts.[1] The actual information on composition and nutritive value of plankton supplied by this investigation is, however, very meager. It seems therefore that no actual data but wishful thinking is responsible for the assumption, discussed in many recent papers, that marine plankton represents a large source of food and that it is only a question of technology of harvesting and of economics to make this material available for practical nutrition.[7,34,40,47,49,51]

In the following we intend to analyze from a nutritional viewpoint the available data in the scientific literature.*

From the Department of Biochemistry and Nutrition, University of Southern California Medical School, Los Angeles, and Van Camp Seafood Co., Terminal Island, California.

* Professor of Biochemistry and Nutrition, University of Southern California School of Medicine.

* A very good review of "The Role of Algae and Plankton in Medicine" has been recently published by M. Schwimmer and D. Schwimmer, Grune and Stratton, Inc., New York, 1955.

Plankton represents a mixture of drifting microscopic plants and small animals which are distinguished as *phytoplankton* and *zooplankton* respectively. The main importance of the phytoplankton is that they serve as good converters of radiating energy to food on which the herbivorous zooplankton thrives.[46] The representatives of phytoplankton are usually too small (5 to 20 mm) to be harvested and most of them cannot be separated even by regular centrifuges. They represent, in fact, a nuisance in harvesting of zooplankton because they clog the holes of the nets.

The zooplankton represents a general category of drifting invertebrate life and not any particular species. The size of the zooplankton varies between 0.5 to 50 mm and the relative participation of the different component species shows considerable geographic and seasonal variations. These changes in the species composition of the plankton will have to be considered in evaluation of the available quantitative data which are based generally on an overall analysis of *some* plankton samples.

Copepods, crustaceans, living on phytoplankton, pelagic tunicates and shrimp-like euphasids are the most important foods for fish and whales. The medusa and the ctenophores present in zooplankton are very voracious animals living on small zooplankton. Others present are syphonophores, satittae, fish eggs, and fish larvae.

The "*dry weight*" of the zooplankton varies between 14, 11, and 5 per cent according to the preliminary treatment such as squeezing, washing with sea or fresh water. The general composition of zooplankton,[20] based on a mixed harvest, is as follows:

	% of dry weight
Protein	52 to 59
Fat	4 to 7
Ash	19 to 32
Carbohydrate (by difference)	13 to 17

The protein content is calculated by multiplying the total nitrogen content by 6.25. We do not know, however, how much of this nitrogen is actually present in available protein. The data on the amino acids present in plankton protein are quite unsatisfactory.[12] Most of the essentials seem to be present but their relative quantities and their biologic availability have not been investigated as yet.[30]

The composition of *fat* is, according to several authors, very similar to the fat extracted from fish.[10] It is characterized by long chain highly unsaturated fatty acids. It is probable, therefore, that these easily oxidizable fatty acids affect the nutritional value of the plankton itself.[50] Some specific *sterols*[52] have been isolated from zooplankton but their biologic effect has not yet been investigated.[2]

The inorganic composition of some species present in zooplankton is well discussed in Vinogradov's monumental work[48] which was recently published in an English translation by Yale University. (A relatively high magnesium, silicon, iodine, iron, and arsenic content is characteristic. Plankton is relatively low in available calcium and sulphur.) (Table I.)

TABLE I
The Average Inorganic Composition

	% of dry weight
Chlorine	14.9
Sodium	21.1
Phosphorus	0.76
Potash	1.42
Calcium	0.96
Magnesium	1.29
Sulphur	0.71

The *vitamin A* content was first investigated by Drummond[13] who found that cod receives its vitamin A through several intermediaries such as copepods, larval decapodes, and mollusca which are present in plankton. The investigation of the actual vitamin A content of zooplankton with the growth test, with the antimony trichloride test, and with the absorption spectrum gave, however, negative results. It was therefore assumed that zooplankton contains some precursors of vitamin A. Recent investigations have shown that all provitamin A activity resides in the non-carotinoid fraction of the plankton oil.[25] This shows that fish use zooplankton pigments other than common carotinoids for the elaboration of vitamin A.[29,33,37] The question arises naturally

whether this material can be used also by mammals for formation of vitamin A.[28]

The *vitamin D* content of plankton,[32] gave a weakly positive result with the line test, but with the x-ray examination a negative result was obtained. It was shown that the vitamin D content of zooplankton was negligible. Provitamin D seems to be present in considerable quantities.[26] The literature on the *niacin*,[24] *vitamin C*, and *vitamin B*[3] content is very meager. We did not find any data on the *vitamin* B_{12} content. Recent investigations show that some marine bacteria are excellent producers of vitamin B_{12} and therefore we may assume that zooplankton may be a good source of this factor.[14]

THE NUTRITIVE VALUE OF PLANKTON

The nutritive value of phytoplankton seems to be somewhat similar to that of grass. Therefore, it does not seem to represent a better potential source of protein than grass grown on pastures. An additional disadvantage is the very high mineral content of the diatomes present in phytoplankton. The preceding indicates that the available data on the composition of zooplankton are not sufficient to draw any conclusions as to its nutritional value. The inference is usually indirect and is based on the so-called "nutritional pyramid" which shows that phytoplankton on the base of this pyramid supplies food for zooplankton, for oysters, mussels, and cockles, and that this herbivorous zooplankton represents then the food for the larger fishes and for the marine mammals.[20] Particularly the fact that some whale varieties that live nearly exclusively on plankton (Krill, *euphasia superba*) grow to 60 feet in length in two years led to the conclusion that zooplankton must be an excellent food for terrestrial mammals also. This conclusion is subject to further investigation because we do not know presently (a) the nutritive requirements of the whale; (b) the particular digestive faculties of this mammal; (c) the efficiency of food conversion.

We do know, however, that the food requirements of terrestrial mammals such as, for instance, that of horses, of men, and of lions are quite different and therefore it does not seem to be permissible at this time to draw far reaching conclusions from the feeding habits of the whale.

Before the Second World War German scientists had claimed that phytoplankton has a nutritive value similar to that of rye flour and that zooplankton is equivalent in its nutritive value to the best meat. Unfortunately no data have been published which would support these occasional claims. As a further proof for the nutritive value of zooplankton, it is usually mentioned that the Chinese and some Scandinavian nations use zooplankton as a base for a tasty paste with a shrimp-like flavor. Finally, Thor Heyerdahl,[23] in his report of the Kon-Tiki voyage, mentioned that zooplankton consisting of copepods, pelagic crabs and other crustaceans, of fish eggs and fish larvae was consumed by some members of the crew. No information is given however on how much of this material was consumed and what other food was eaten at the same time. These reports therefore show only that zooplankton *can* be eaten but do not say anything about its nutritional value or about its effect on the digestive organs when consumed chronically in considerable quantities.

The only approach to evaluate the nutritive value of zooplankton experimentally has been attempted by Clarke and Bishop.[9] These authors investigated the growth and survival of weanling rats for a period of 13 days and of adult rats for a period of 22 days. The plankton used was obtained from the Vineyard Sound and was frozen at $-18°$ C after reaching the laboratory. It was squeezed with moderate pressure to remove excess sea water and contained about 14 per cent of dry material. The diet mixtures were prepared on the basis of the dry weight. As control feed Purina-Growena was used. Rats fed on plankton only lost weight rapidly and died within 4 to 19 days, but survived about 30 per cent longer than controls on total starvation. Rats fed on a diet composed of two-thirds meal and one-third plankton and two-thirds plankton and one-third meal failed to grow as fast as rats on a full diet. The authors do not present detailed data on food consumption but state only that the rats ate only about two-thirds of the ration

offered when it contained plankton. They, therefore, arrive at the conclusion that the rats derived some nourishment from plankton but were able to assimilate only a small fraction of it. They observed also that the stomach of the animals contained undigested material and that fecal material was congested in the cecum and in the "hind" intestine. They investigated also the effect of plankton consumption in man. About these experiments they write as follows:

"No toxic effects developed after eating 100–200 grams (wet weight) of the material during the course of a day. The maximum quantity eaten by any one of the subjects at one time was 100 grams. Larger amounts were definitely unacceptable and distasteful. It is probable that psychological factors entered into the matter of the palatableness and digestibility of the plankton in the laboratory and at sea. Such factors are not easy to evaluate. It was certainly true that a quantity of only 30–40 grams of plankton gave the impression of remaining undigested in the stomach for several hours after eating."

Clarke and Bishop performed these experiments with particular observation of life raft conditions and came to the following conclusions:

"Plankton with the observed chemical composition was calculated to have an approximate maximum energy content of 4 cal./g. dry weight. The 2400 cc. of plankton which could theoretically be obtained from a life raft in 24 hours would thus provide 788 calories, if it could all be assimilated, or about 1/4 of a man's average daily requirements."

A critical review of Clarke and Bishop's results shows that their methods were not up to the present standards by which nutritional experiments should be designed. Some of the objections are that the rats consumed much smaller quantities from the diet containing plankton than from the control diet. Furthermore, we see that due to the high water content (8 per cent) of the experimental diet, too large quantities have been offered. The high water content of the diet may lead to an abnormal dilution of the digestive juices. The high salt content of the diet may have influenced the utilization. Furthermore, large quantities of undigested material were stagnating in the intestinal tract. This may have influenced not only the utilization of the diet itself but also the important nutritional function of the intestinal flora. It is quite amazing that Clarke and Bishop's experiments were widely publicized without pointing out the weak points in their experimental approach. It is surprising also that no other fundamental investigations on the nutritive value of zooplankton have been published. In our opinion, the experiments of Clarke and Bishop do not supply enough material to draw any conclusion as to the food value of zooplankton.

ECONOMIC PROBLEMS

A not negligible problem seems to be the economics of zooplankton collection. Before this question and the technical approach of harvesting can be seriously considered, the following matters will have to be clarified: first, whether zooplankton can be used directly for human nutrition or indirectly by feeding it to poultry and to other livestock. In this connection we will have to consider that presently large quantities of low-priced protein supplements with high biologic value are available such as fish meal, meat scrap, or blood meal. We also have to realize that the chemical industry recently made some essential vitamins and amino acids available at prices which are generally much lower than the cost of production from natural material.

A further problem is one of locating which are the best harvesting grounds and the best seasons to collect zooplankton.[6,42,43] The variability of plankton[11] is such that it would be exceedingly difficult to predict what type and what amount would be encountered in any given situation, at least until that area has been studied for a long time.[19,38] It was found by several authors that the distribution of zooplankton[27,44] is patchy, that there are abrupt changes in composition as well as in amounts, that there is a *vertical diurnal variation* so that some species climb several hundred feet in the evening toward the surface of the ocean. Besides this, there is the recently discovered *seasonal vertical variation* in plankton distribution. Also recently described as an important source of zooplankton is the *scattering layer*[22] which exists between 900 and 2,700 ft and approaches the surface between sunset and sunrise and which consists mainly of small crus-

taceans. We have to realize furthermore that the growth of zooplankton depends on the available phytoplankton.[31,35,36,51] The phytoplankton[15,16] needs sunshine and minerals for its development and if the minerals are exhausted, or if the water is not plowed, i.e., if the minerals are not replaced from the deeper layers, the phytoplankton dies.[21,39] This mixing with deeper water or with river water occurs at the shallow coasts of West Africa and the western South American coast.[35] Investigations in the North Atlantic show a main flowering in spring and waning of the population in summer and a second crop in autumn or winter. Not well explained, however, is the abnormally high density in the high latitudes near the Poles.

The question as to the feasibility of collecting plankton has been discussed by scientists and lay authors and we have to realize that both usually go "overboard" in their predictions.* Some authors, for instance, claim that the sea yields from one to three tons of organic material per acre per year. This means about as much as one acre of forest. Because the ocean represents about 70 per cent of the earth's surface the sea produces about twice as much as the land.[42] Other authors, however, do not agree with these calculations, and Nielson assumes that plants in the ocean fix 12 million tons of carbon yearly compared with the 19 million tons fixed by land plants.[39,47] Most of these estimates include phyto and zooplankton. The actual yield on zooplankton was experimentally determined by Bigelow and Sears[4] who found in the upper water layers during maximal production 0.5 to 0.8 ml zooplankton per m^3 of water. Clarke and Bumpus found in the shallow stratum 0.3 to 1.9 ml.[8] Heyerdahl obtained during one day 2.5 to 5 kg of zooplankton. Hardy[20] predicts that with suitably constructed nets two men could collect daily 588 lb of plankton on the coasts of Scotland "enough to feed 357 people." We think however, that it should be added: "provided that people would eat, digest, and utilize the zooplankton." A further complication seems to be that zooplankton cannot be harvested without admixture of some phytoplankton.

The collection of phytoplankton always represents a hazard because there are different highly toxic representatives known, for instance *Gonyaulax catenella* in the California waters and Gymnodium dinoflaggalates which produce extensive fish mortality.[5] We have to assume that there are many other toxic plankton forms living in different geographic locations which may lead to poisoning of fish or which turn otherwise edible fish and seafood into a highly toxic food.[18] Another danger in consumption of plankton may be the swallowing of stinging forms as for instance the consumption of Portuguese Man-O-War may have serious consequences.

We want to emphasize that instead of harvesting zooplankton it would be more realistic and more economical for the time being to increase the catch of herring-varieties, of manhaden and "trashfish." This could be done easily provided the human demands for this nutritious food were augmented. In many countries where the animal protein is not available in sufficient quantities, the most urgent problem seems to be the preservation of the fishcatch in palatable form ready for transportation from the fishing areas to the interior.[41]

The reluctance to accept fishflour as food ingredient for human consumption, as observed recently in many "underprivileged" countries, in my opinion, raises a serious doubt as to whether dried zooplankton will ever be acceptable as protein supplement for human nutrition.

It seems that first the problems connected with the nutritive value of plankton and the feasibility of feeding it to men and animals must be solved, and then the different harvesting territories will have to be investigated for possible contamination of the crop with toxic material. This latter problem seems of minor importance, however, because of the geographic and seasonal limitation of toxic forms.

A further problem in plankton collection is the development of efficient harvesting and

* It was suggested recently, in an article based on sheer speculation, that trawling of krill in the Antarctic area, may be more profitable than whaling and may help to solve the world's food protein shortage. (W. E. Pequegnat: *Scient. Amer.* 198: 84, 1958.)

drying equipment.[45] Recently excellent rotary filters and driers have been developed for other purposes and it seems that such equipment could be well adapted for plankton harvesting. The final step in connection with our problem seems to be the evaluation of the economics of plankton collection in dollars and cents. Our present abundance of food resources seems to be responsible for the fact that this question has never been actually investigated. Until now there has not been an urgent necessity to solve or even seriously study these problems, but we quote in this connection the following statements made by Lucas: "In the presence of certain alternatives there may not be the incentive to attempt to solve the technical problems which might easily—and profitably—be solved if these alternatives were no longer. The result might be a much cheaper source of food, in far greater quantity, than those whose abundance originally discouraged the urge to tap a new source."

SUGGESTIONS FOR FURTHER NUTRITIONAL RESEARCH

Before any economic or technical problems connected with the utilization of plankton are seriously considered, the question should first be investigated whether plankton can be used as such or as a supplementary food for human feeding or for livestock nutrition. In order to clarify these points the amino acid composition and the digestibility of plankton protein will have to be investigated.

Animal experiments may give information on the biologic value of this protein and on its efficiency as a supplementary food. The possible presence of "unidentified" growth factors may be studied and also the question whether the provitamins present in plankton which can be utilized by fish have the same nutritional importance in mammals and birds. It will also be necessary to study how the excess of minerals and particularly of some cations such as magnesium can be eliminated.

The nutritive value of crops harvested at different localities should be compared.

Finally, as a crucial experiment, plankton should be fed to man under well controlled conditions, controlling the utilization of plankton protein by nitrogen balance experiments.

These investigations may be time consuming and expensive but they would yield some basic answers to our problem. As such, they would represent a great service not only to the science of nutrition but also to humanity because they may answer the question of whether there is a short cut possible in the nutritional pyramid, i.e., whether plankton can be utilized directly for feeding of man and terrestrial animals. There is a possibility that the utilization of plankton for such purposes may not be feasible and that we will have to rely in the future also on intermediary food converters in the form of fish and other conventional seafoods which convert the nutrients present in plankton to a form which is acceptable to and can be utilized by man and terrestrial animals.

REFERENCES

1. Bajikov, A.: Utilization of marine plankton as food in emergency. *Army Air Force Serial No.* T SEAL-5H-4-184, 1945.
2. Belloc, G., Fabre, R., and Simmonet, H.: The study of the biological activity of sterols. The study of sterols of plankton. *Compt. rend.* 191: 160, 1930.
3. Van Beneden, G.: Presence of vitamin B and C in plankton. *Bull. centre Belge etude et document eaux* (Liege) II: 216, 1949.
4. Bigelow, H. B. and Sears, M.: *Harvard University. Museum of Comparative Zoology. Memoirs.* 54: 189, 1939.
5. Bonnot, P. T. and Phillips, B.: Red water. *Calif. Fish and Game* 24: 57, 1938.
6. Chace, F. A.: Plankton of the Bermuda Oceanogr. Exped. IX. *Zoologica* (N. Y.) 25: 117, 1940.
7. Clarke, G.: Discussion: Plankton as a food source for man. *Science* 89: 602, 1939.
8. Clarke, G.: *Elements of Ecology.* Wiley, New York, 1954.
9. Clarke, G. L. and Bishop, D. W.: The nutritional value of marine zooplankton. *Ecology* 29: 54, 1948.
10. Collin, G., Drummond, J. C., Hilditch, T. P., and Gunther, E. R.: Observations on the fatty constituents of marine plankton. *J. Exper. Biol.* 11: 198, 1934.
11. Corlett, T.: Net phyto-plankton at Ocean Weather Stations I and J. *J. du Conceil* (Copenhagen) 19: 178, 1953.
12. De la Torre, A. M. C.: Methionine and cystin in some crustaceans. *An. Fac. Farm. Broc. Lima* (Lab. Control Inst. Sanit., Lima) 141, 1952–53.
13. Drummond, T. C. and Gunther, G. R.: Vitamin

content of marine plankton. *Nature* 126: 398, 1930.

14. ERIKSON, L. and LEWIS, K.: Vitamin B_{12} in algae. *Ark. Kemi* (Stockholm) 6: 427, 1953.
15. GRAHAM, H. W.: The phytoplankton. *Sci. Res. Cruise VII, Carnegie Inst. Washington Publ.* 1–10, 1943.
16. GRAHAM, H. W.: Plankton production in relation to character of water in the open Pacific. *J. Mar. Res.* 4: 189, 1941.
17. HABERLING, W.: *Johannes Müller. . .* Akad. Verlagsgesellschaft, Leipzig, 1924.
18. HALSTEAD, B. W. and BUNKER, N. C.: Poisonous fishes of Johnston Island. *Zoologica* (N. Y.) 39: 6, 1954.
19. HARDY, A. C.: Some problems of pelagic life; in *Essays in Marine Biology* (ed. S. M. Marshall and A. P. Orr). Oliver & Boyd, Edinburgh, 1953.
20. HARDY, A. C.: Plankton as a source of food. *Nature* 147: 695, 1941.
21. HARVEY, H. W. T.: Substances controlling the growth of diatoms. *J. Mar. Biol. Assoc. Unit. Kingdom* 23: 499, 1939.
22. HERSEY, T. B., JOHNSON, H. B., and DAVIS, L. C.: Recent findings about the deep scattering layer. *J. Mar. Res.* 11: 1, 1954.
23. HEYERDAHL, T.: *Kon-Tiki.* Rand-McNally, Chicago, 1950, pp. 138–141.
24. HUTCHISON, G. E. and SETLOW, J. K.: Niacin cycle in a small inland lake. *Ecology* 27: 13, 1946.
25. JAMESON, H. L., DRUMMOND, J. C., and COWARD, K. H.: Synthesis of vitamin A by a marine diatom (*Nitzschia closterium* W. Sm.) growing in pure culture. *Biochem. J.* 16: 482, 1922.
26. JOHNSON, N. G. and LEVRING, T.: Production of vitamin D in the sea. *Svenska Hidrog. Biol. Komm. Skrifter Ser.* 3: 1, 1947.
27. JUDAY, C.: The utilization of aquatic food resources. *Science* 97: 456, 1943.
28. KON, S. K.: Vitamin A in marine invertebrates and the metabolism of carotinoids of plankton. *Bull. Soc. Chim. Biol.* 36: 209, 1954.
29. LANE, C. E.: A noncarotene provitamin A for fishes. *Science* 111: 471, 1950.
30. LASKER, R. and LANE, C. E.: The origin and distribution of nitrogen in *Teredo bartschi. Biol. Bull.* 105: 316, 1953.
31. LEBRASSEUR, R. T.: Zooplankton distribution in the northeast Pacific Ocean. *Fisheries Research Board of Canada. Prog. Rep.* 107: 3, 1956.
32. LEIGH-CLARE, J. L.: Search for vitamin D in the diatom *Nitzschia closterium* (W. Sm.). *Biochem. J.* 21: 368, 1927.
33. LOVERN, J. A.: Vitamin A from astaxantin. *Chem. and Indus.* 61: 222, 1942.
34. LUCAS, G. E.: Harvesting the waters; in *Four Thousand Million Mouths.* (ed. F. LeGros Clark and N. W. Pirie.) Oxford Univ. Press, Toronto, 1951.
35. MARSHALL, S. M. and ORR, P.: Production of animal plankton; in *Essays in Marine Biology.* Oliver & Boyd, Edinburgh, 1953.
36. MARSHALL, N. B.: *Aspects of Deep Sea Biology.* Philosophical Library, New York, 1954.
37. MCWALTER, R. J. and DRUMMOND, J. C.: The relation between lipochrome pigments and vitamin A in the nutrition of young fish. *Biochem. J.* 27: 1415–20, 1953.
38. NIELSEN, E. S.: Die Ceratien des Indischen Ozeans und der Ostasiatischen Gewasser. *Dana Report* (Copenhagen) 17, 1939.
39. NIELSEN, E. S.: Production of organic matter in the sea. *Nature* 169: 956, 1952.
40. PYKE, M.: *Townsman's Food.* Turnstile Press, London, 1952.
41. REAY, G. A.: The ocean as a potential source of world food supply. *Food Tech.* 8: 65, 1954.
42. RILEY, G. A.: Letter to the Editor: Arguments against Nielsen on the fertility of the sea. *J. du Conceil* (Copenhagen) 19: 85, 1953.
43. RILEY, G. A., STOMMEL, H., and BUMPUS, D. F.: Quantitative ecology of plankton of the western North Atlantic. *Bull. Bingham Oceanogr. Coll.* 12: 1, 1950.
44. SEWELL, R. B. S.: The free swimming planktonic copepoda. *Brit. Mus. Nat. Hist. Geographical Distrib. John Murray Exped. Sci. Rep.* 8: 1948.
45. SHROPSHIRE, R. F.: Plankton harvesting. *J. Mar. Res.* 5: 185, 1954.
46. SMITH, F. G. W. and CHAPIN, H.: *The Sun, the Sea and Tomorrow.* Scribners, New York, 1954.
47. STEEMAN, N.: Production of organic matter in the sea. *Nature* 169: 956, 1952.
48. VINOGRADOV, A. P.: *Elementary Chemical Composition of Marine Organisms.* Sears Foundation for Marine Research, Yale University, New Haven, 1953.
49. WEISS, F. J.: Food from the sea. *J. Agric. Food Chem.* 1: 823, 1953.
50. WELCH, P. S.: *Limnological Methods.* Blakiston, Philadelphia & Toronto, 1948.
51. WILLIAMS, T. I. (ed.): *The Soil and the Sea: A Symposium.* Saturn Press, London, 1949.
52. YOSHIYUKI, T., KITA, M., and TANAKA T.: Sterols in the fat of small fishes. *Bull. Chem. Soc. for Japan* 25: 355, 1952.

Nutritional Status of Selected Adolescent Children

III. ASCORBIC ACID NUTRITURE ASSESSED BY SERUM LEVEL AND SUBCLINICAL SYMPTOMS IN RELATION TO DAILY INTAKE

MARGARET MCGREGOR HARD, M.S.,* NETTIE C. ESSELBAUGH, PH.D.,* AND ELIZABETH A. DONALD, M.S.†

ADOLESCENCE is a period of great physiologic stress. Even so, this age has received comparatively little attention from the viewpoint of basic nutritional information. Some adolescent subjects have been included in ascorbic acid studies involving children of various ages.[1–9] A few studies have emphasized the 14-to 16-year-olds.[10–12] The main methods for assessing dietary adequacy and ascorbic acid nutrition have been dietary records and blood level of ascorbic acid.

In the course of a comprehensive study of 248 selected adolescent boys and girls in two areas of Washington State,[13] information was obtained pertaining to ascorbic acid status. Dietary intake, serum ascorbic acid, biomicroscopic examination of the gums and of the epithelium of the upper arm, colored photography of the gums, and physical examination of the gums and of the skin of the upper arm were the indices available for the evaluation of ascorbic acid nutrition. In this report, correlations between food intake, serum ascorbic acid levels, and physical manifestations of ascorbic acid deficiency are discussed as well as the ascorbic acid status of the selected adolescent subjects.

Scientific Paper No. 1666, Washington Agricultural Experiment Stations, Pullman. Project 1013.

Supported in part by regional W-4 9b3 funds obtained under the Research and Marketing Act, and cooperative with the Human Nutrition Research Branch, Agricultural Research Service, United States Department of Agriculture.

* Associate Home Economists.

† Acting Junior Home Economist.

PROCEDURE

The selection of areas for study and the description of subjects have been reported previously.[13] Yakima County, east of the Cascade Mountains and Snohomish County, west of the Cascades, were the two areas chosen. The subjects were 15- and 16-year-old boys and girls who had been born and reared in either of these counties. About 125 subjects were studied in each of the two areas. There were almost equal numbers of boys and girls in each age group in each area.

Dietary Data

Semiquantitative seven-day dietary records[13] were the source of the information on ascorbic acid intake from food. Vitamin supplements were recorded separately.

Serum Ascorbic Acid

Serum ascorbic acid determinations by the method of Lowry, Lopez, and Bessey[14] were made on fasting blood immediately following collection.

Biomicroscopic Examination

Examination of the gingiva and of the epithelium of the upper arm was made with the Bausch and Lomb Poser Slit Lamp. All biomicroscopic observations were made by the same operator.

The Gingiva: Hyperemia, swelling, infiltration, pitting, recession of the interdental papillae, and recession at the dental margin were noted in the gingiva of the upper and lower gums. A qualitative value of "normal," "slight," "some," "marked," or "very marked" was assigned to each lesion. Each qualitative description was arbitrarily quantified by giving "normal" the value of 1; "slight," 2; "some," 3; "marked," 4; and "very marked," 5. The average value was obtained for the entire gum for each subject because the upper or lower gingiva is not likely to be more affected than the other.[15] The presence of any one of the symptoms was classified as a chronic condition. The value for hyperemia plus swelling was obtained in addition to the above values and was considered to be an acute condition.

The Epithelium of the Upper Arm: The posterolateral aspect of the left upper arm was examined for signs of hyperemia. This lesion was qualitated and quantified in the same manner as the gingiva.

Colored Photography

The gums of each subject were photographed using a Leica camera with 135 mm telephoto lens, 90–100 mm extension tube and ground glass copy attachment for focusing, Kodachrome A film, a 81C Rattan filter and #6 G.E. flash bulb. The distance of camera from subject was 11 inches. Each slide was evaluated as "good," "fair," or "poor" for each of the lesions, hyperemia, swelling, recession of the interdental papillae, and recession of the dental margin. These qualitative values were arbitrarily assigned quantitative values of "good," 1; "fair," 2; and "poor," 3. The lesions of each subject were further evaluated as acute or chronic. The colored-slide evaluation and biomicroscopic examinations were made by the same operator. Viewing distance from the 54 × 40 inch screen was 9 feet.

Physical Examination

The epithelium of the upper arm and the gums were examined during the physical examination of each subject. One physician made the examination in Snohomish County, a second physician in Yakima County. Perifollicular petechiae (hyperemia) of the arm were observed as "mild," "moderate," or "severe." Gingivitis (swelling), recession of the interdental papillae, recession at the dental margin, and hyperemia were noted in the gums. The quantitative values for the qualitative descriptions were "absent," 1; "mild," 2; "moderate," 3; "severe," 4.

Statistical Treatment of Data

Daily mean intakes of ascorbic acid and standard error of the means were obtained for both boys and girls from Snohomish and Yakima Counties. The arithmetic mean intakes were compared with the National Research Council Recommended Allowances.[16] Since skewness was apparent, the geometric means and their logarithms were used in statistical treatments involving daily intake data.

Blood serum ascorbic acid and the quantified values for the biomicroscopic observations, the colored photography evaluations and the physical examinations were expressed in terms of mean values for Snohomish County boys and girls and Yakima County boys and girls.

Analysis of variance was used for comparisons between groups.[17]

Correlations[17] were calculated for the four groups: Snohomish boys, Snohomish girls, Yakima boys, and Yakima girls. Ascorbic acid intake including vitamin C supplementation was correlated with serum ascorbic acid levels and with the various quantified physical and biomicroscopic values. Similarly, the quantified values were correlated with each other and with the serum levels.

RESULTS AND DISCUSSION

Ascorbic Acid Intake

Ascorbic acid intakes from food were low for all groups except the Snohomish girls (Table I). Thirty-two per cent of the girls were getting less than 50 mg per day, while 34 per cent of the boys had less than 60 mg daily.[13] The girls in Yakima County consumed significantly less ascorbic acid (at the 1 per cent level) than the Snohomish girls. No significant difference was found between the intakes of 15- and 16-year-olds, either boys or girls.

When the daily mean intakes were compared with the National Research Council Recommended Allowances,[16] the Snohomish girls and boys consumed 99 and 87 per cent, respectively, of the recommended ascorbic acid allowances and the Yakima girls and boys, 74 and 88 per cent, respectively. Other dietary studies[18–22] have indicated that ascorbic acid may be low in the diets of adolescent children.

Fourteen of the 248 subjects reported the intake of vitamin C supplements. For 6 of the 14, supplementation considerably increased (by 17 to 1666 per cent) the intakes from diets alone.

Blood Serum Ascorbic Acid

The serum ascorbic acid levels (Table I) rate "good" for the girls and "fair to good" for the boys as compared with the serum ascorbic acid classification reported by Bessey and Lowry.[23] No significant differences were found between 15- and 16-year-olds or between areas. A significant difference (at the 5 per cent level) existed between sexes. Sex differences have been noted also by Bring, Warnick, and Woods[11] in Idaho, and by Clayton *et al.*[24] in the Northeast.

One-fourth of the girls and over one-third of the boys had serum levels of 0.4 mg per 100 ml or less. Twenty-seven per cent of the girls also had serum ascorbic acid levels of 1.1 mg per 100 ml or over while only 15 per cent of the boys reached this value. A similar distribution has been reported by Roderuck *et al.*[7] Clayton *et al.*[24] found a lower percentage of males than females in the 1.0 mg per 100 ml groups, as did Wilcox *et al.*[1] in the Utah 13- to 19-year-old nonrheumatic subjects. Storvick, Hathaway, and Nitchals[10] on the other hand, found about one-third of both Oregon girls and boys in the "excellent" range. Montana adolescents[12] had higher serum ascorbic acid levels; 73 per cent of the girls and 58 per cent of the boys could be classified as "excellent."

That the serum ascorbic acid levels of adolescent subjects are directly related to the food intake is illustrated in Figures 1 and 2. The correlation coefficient (r) was 0.616 for all subjects (significant at the 1 per cent level), 0.683 for boys, and 0.543 for girls. It has been reported[7] that ascorbic acid intake per kilogram of body weight is a more accurate indicator of serum concentration than is daily ascorbic acid intake. A relationship between intake per kilogram and serum concentration did exist in both adolescent boys and girls. However, the correlations between intake per kilogram and serum concentration were less ($r = +0.674$ for the boys and $r = +0.470$ for the girls) than the correlations with daily ascorbic acid intake.

There appears to be a difference in ascorbic acid metabolism in girls compared to boys. Although the daily ascorbic acid intake tended to be lower for the girls than for the boys (Table I), the serum blood levels of the girls were significantly higher than those of the boys. It is difficult to draw definite conclusions on the basis of a week's intake and of one blood sample, but the blood levels for the girls do seem higher than might be expected. This has been reported also by Wilcox *et al.*[1] and by Roderuck

TABLE I

Mean Daily Intake of Ascorbic Acid and Mean Serum Levels of Ascorbic Acid by Area and Sex

	Snohomish County		Yakima County	
	Boys (63)	Girls (62)	Boys (61)	Girls (61)
	Daily intake			
Arithmetical mean (mg) and S. E. of mean	82 ± 5.73	79 ± 5.33	83 ± 5.00	59 ± 3.97
Geometric mean (mg)	72	69	74	52
Log. of geom. mean and its S. E.	1.8550 ± 0.0287	1.8372 ± 0.0308	1.8710 ± 0.0271	1.7186 ± 0.0285
	Serum levels			
Mean (mg/100 ml) and S. E.	0.64 ± 0.04	0.86 ± 0.06	0.70 ± 0.06	0.72 ± 0.06

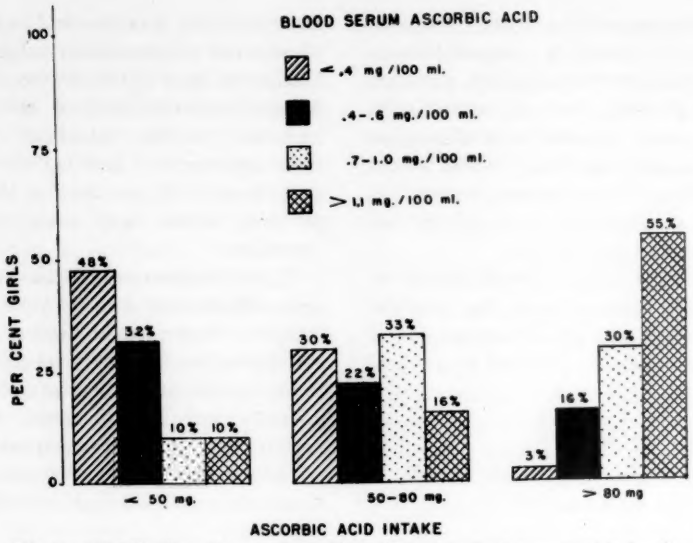


Fig. 1. Distribution of serum ascorbic acid levels of adolescent girls having daily ascorbic acid intakes of <50 mg, 50–80 mg, and >80 mg.

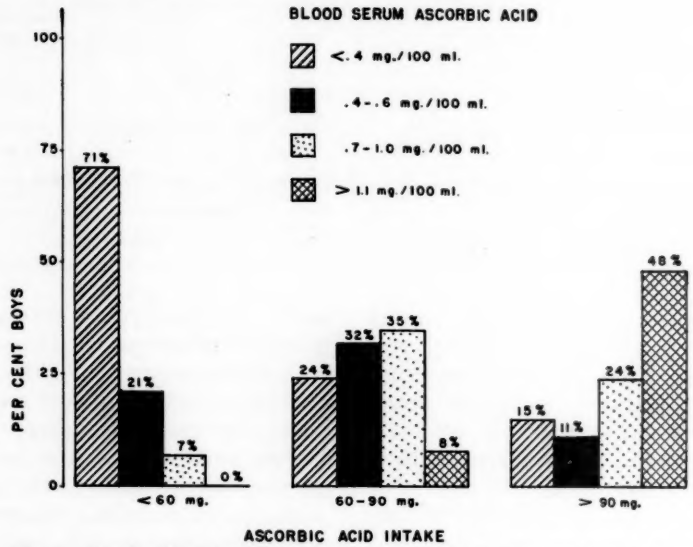


Fig. 2. Distribution of serum ascorbic acid levels of adolescent boys having daily ascorbic acid intakes of <60 mg, 60–90 mg, and >90 mg.

et al.[7] On the hypothesis that the sex difference might be due in part to hormonal influence, a correlation was made between basal metabolic rate and serum ascorbic acid. Only one group, the Yakima boys, showed significant relationship (at the 5 per cent level). Tisdall and Jolliffe[25] have suggested an association of ascorbic acid deficiency with increased respiratory rate.

Subclinical Symptoms of Ascorbic Acid Deficiency

Biomicroscopic Examination: Biomicroscopic observations of subclinical symptoms often

associated with ascorbic acid deficiency are summarized in Table II. The average quantified scores for the arm and gingival lesions indicate a "slight" deficiency of vitamin C. Gingival recession is somewhat more pronounced than the other manifestations. The scores for chronic lesions (column 8) were somewhat higher for all subjects than were those for acute lesions (column 7). This suggests a greater incidence of chronic than of acute gum lesions. Except for hyperemia of the arm and infiltration of the gingiva, a greater percentage of the girls than of the boys were "normal."

It is not possible to make comparisons among the studies which have used biomicroscopic observations to assess nutritional status because many different methods of describing the lesions and quantifying the results have been employed.

Simple correlation coefficients for acute and chronic gingival lesions and hyperemia of the arm with serum ascorbic acid and with ascorbic acid intake with supplements are given in Table III. All were nonsignificant except for chronic gingival lesions and serum ascorbic acid in Yakima girls. This correlation ($r = -0.263$) significant at the 5 per cent level, may be attributable to chance.

The lack of correlation between dietary intake, blood values, and biomicroscopic observations is in agreement with the theory proposed by Kruse.[26] Serum ascorbic acid values fluctuate with current intake whereas the state of body tissues probably reflects the nutritional status of an individual over a period of *years*. Biomicroscopic observations of the tissues made by an experienced operator should contribute information to the over-all assessment of nutritional status.

Kodachrome Observations: The average scores of the gingival lesions (Table IV) as observed from kodachrome slides generally agree with the biomicroscopic results. The evaluation of the slides is not as precise a measure of subclinical symptoms as is the biomicroscopic examination. This is reflected in the quantifying scale—the biomicroscopic observations were quantified from 1 to 4, but the kodachrome scale was 1 to 3. On this basis, the kodachrome observations suggest a "fair" state of ascorbic acid nutrition in these subjects.

Table III reveals a highly significant correlation between the kodachrome and biomicroscopic observations for acute and chronic gingival lesions. This suggests, that under

TABLE II

Mean Quantified Scores* for Biomicroscopic Observations of the Gingiva and of the Epithelium of the Upper Arm

Tissue	Gingiva																Arm	
Lesions	Hyperemia (1)†		Swelling (2)		Infiltration (3)		Pitting (4)		Recession of I.D.P.‡ (5)		Recession at D.M.‡ (6)		Acute Lesions (1 & 2) (7)		Chronic Lesions (1–6) (8)		Hyperemia (9)	
	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"	Score	"Normal"
Snohomish		%		%		%		%		%		%		%		%		%
boys (63)	1.6	56	2.0	18	2.4	4	2.2	8	2.8	4	2.3	19	1.8	37	2.2	18	1.2	86
Snohomish																		
girls (62)	1.4	62	1.9	28	2.4	4	2.1	12	2.6	5	1.8	34	1.6	45	2.0	24	1.2	76
Yakima																		
boys (61)	1.4	64	1.8	34	2.4	14	2.1	5	2.8	5	2.7	12	1.6	49	2.2	22	1.0	98
Yakima																		
girls (62)	1.4	62	1.7	36	2.3	14	2.2	2	2.5	9	2.1	25	1.6	49	2.0	25	1.1	90
All boys	1.5	60	1.9	26	2.4	9	2.2	6	2.8	4	2.5	16	1.7	43	2.2	20	1.1	92
All girls	1.4	62	1.8	33	2.4	9	2.2	7	2.6	7	2.0	30	1.6	47	2.0	24	1.2	83

* 1, "normal"; 2, "slight"; 3, "some"; 4, "marked"; 5, "very marked."

† Column number.

‡ I.D.P., Interdental papillae; D.M., Dental margin.

TABLE III

Simple Correlation Coefficients for Biomicroscopic Observations

Biomicroscopic observations	County	Sex	Ascorbic Acid		Gingiva lesions			Arm
					Acute	Chronic		Hyperemia
			Intake	Serum	Kodachrome	Kodachrome	Physical	Biomicroscopic
Acute gingiva	Snohomish	Boys	−0.154	−0.100	+0.488†	+0.523†	+0.333†	−0.032
		Girls	+0.081	+0.064	+0.287†	+0.378†	—	+0.021
	Yakima	Boys	−0.101	−0.044	+0.663†	+0.742†	+0.737†	+0.100
		Girls	−0.206	−0.165	+0.747†	+0.786†	+0.533†	+0.113
Chronic gingiva	Snohomish	Boys	−0.053	+0.007	+0.530†	+0.611†	+0.457†	+0.196
		Girls	+0.012	+0.144	+0.219	+0.368†	—	−0.159
	Yakima	Boys	−0.152	−0.048	+0.392†	+0.569†	+0.588†	+0.122
		Girls	−0.219	−0.263*	+0.461†	+0.573†	+0.482†	−0.133
Hyperemia of arm	Snohomish	Boys	−0.206	−0.086	−0.073	+0.010	+0.133	—
		Girls	−0.012	−0.160	−0.054	−0.008	—	—
	Yakima	Boys	−0.004	+0.045	+0.142	+0.179	+0.184	—
		Girls	+0.013	+0.206	—	−0.014	+0.001	—

* Significant at 5% level.
† Significant at 1% level.

conditions of very good photography and in studies where precise results are not required, the evaluation of kodachrome slides for subclinical symptoms of ascorbic acid deficiency may be substituted for biomicroscopic examination. They have the advantage of providing a more objective evaluation of subclinical symptoms than does a physical examination and they also give a clinical record of the tissue for future reference. Merrow *et al.*[27] have made similar observations. As may be expected no correlation was found between kodachrome observations of chronic gingival lesions and ascorbic acid intake ($r = -0.226$) or serum ascorbic acid ($r = -0.157$).

Physical Examination: A higher incidence of gingival involvement was noted among the Yakima than among the Snohomish subjects. This may be due to differences in evaluating symptoms by the two examining physicians. No abnormalities were recorded for the Snohomish girls. "Mild" recession of the interdental papillae was reported for 10 per cent of the Snohomish boys. In Yakima County, a highly significant correlation was noted between the physical and biomicroscopic scores for acute and chronic gingival lesions (Table III). Similarly, the correlation between physical and kodachrome observations in Yakima County were significant at the 1 per cent level

TABLE IV

Mean Quantified Scores* for Kodachrome Observations of the Gingiva

	Hyperemia (1)†	Swelling (2)	Recession of I.D.P.‡ (3)	Recession at D.M.‡ (4)	Acute lesions (1 & 2) (5)	Chronic lesions (1–4) (6)
Snohomish boys (61)	1.7	2.2	1.6	1.6	2.0	1.8
Snohomish girls (62)	1.9	2.0	1.6	1.5	2.0	1.8
Yakima boys (61)	1.9	2.1	1.9	1.9	2.0	2.0
Yakima girls (62)	1.8	2.2	1.7	1.7	2.0	1.8
All boys	1.8	2.1	1.8	1.8	2.0	1.9
All girls	1.9	2.1	1.7	1.6	2.0	1.8

* 1, "good"; 2, "fair"; 3, "poor."
† Column number.
‡ I.D.P., Interdental papillae; D.M., Dental margin.

($r = +0.640$ for the boys, $r = +0.626$ for the girls). The average scores for acute lesions as observed by the physician were 1.3 and 1.4 for Yakima girls and boys, respectively, and 1.3 and 1.3 for the chronic lesions. About one-fourth of the Yakima subjects were noted to have "mild" to "moderate" manifestations of swelling, recession of the interdental papillae and recession at the dental margin.

The various methods used in this study to assess ascorbic acid nutrition agree fairly well. From the standpoint of present recommendations, the daily food intake is considered to be low. The serum ascorbic acid levels can be classified as "fair" to "good." The subclinical symptoms of deficiency were observed to be "slight." Taking all these criteria into consideration, the ascorbic acid nutriture of the selected Washington adolescent subjects can be described as fairly good.

SUMMARY

The ascorbic acid nutrition of 248 adolescent boys and girls born and reared in two areas of Washington (Snohomish and Yakima Counties) was assessed by five methods—dietary intake including supplementation, serum blood levels, biomicroscopic and kodachrome evaluation of subclinical deficiency manifestations, and physical examination.

By the present standards the ascorbic acid food intake of all boys and of the Yakima girls was low. Mean daily intakes were 82 mg for the boys and 69 mg for the girls. Few (14) subjects took vitamin C supplements.

The mean serum ascorbic acid values were rated "fair" to "good." The values for Snohomish boys and girls were 0.64 ± 0.04 and 0.86 ± 0.06 mg per cent, respectively, and for the Yakima boys and girls, 0.70 ± 0.06 and 0.72 ± 0.06 mg per cent, respectively. A significant difference existed between the sexes.

The average scores for the biomicroscopic observations of the upper arm and gingival epithelial lesions indicated a "slight" deficiency of ascorbic acid. The evaluation of the kodachrome slides suggested a "fair" status of ascorbic acid nutrition. The physical examination revealed few subclinical manifestations.

On the basis of the several criteria used, selected Washington adolescent subjects may be described as being in a fairly good state of ascorbic acid nutrition.

REFERENCES

1. Wilcox, E. B., Galloway, L. S., Wood, P., and Mangelson, F. L.: Children with and without rheumatic fever. III. Blood serum vitamins and phosphatase data. *J. Am. Dietet. A.* 30: 1231, 1954.
2. Moyer, E. Z., Harrison, A. P., Lesher, M., and Miller, O. N.: Nutritional status of children. III. Blood serum vitamin C. *J. Am. Dietet. A.* 24: 199, 1948.
3. Lindgren, I.: Ascorbic acid content of the blood of school children during the spring and the fall. *Am. J. Dis. Child.* 73: 209, 1947.
4. Cooperstock, M., Morse, E., Moyer, E. Z., and Macy, I. G.: Nutritional status of children. IV. Nutritional conditioning in a health camp. *J. Am. Dietet. A.* 24: 205, 1948.
5. Crane, M. M. and Wood, P. W.: A study of vitamin C nutrition in a group of school children. *Am. J. Dis. Child.* 64: 151, 1942.
6. Hjarne, U.: Seasonal variations of the vitamin C content of the blood. *Am. J. Dis. Child.* 73: 88, 1947.
7. Roderuck, C., Pudelkewicz, C., Emil, H. J., and Eppright, E. S.: Serum vitamin C of Iowa school children and its relationship to diet and age. *J. Nutrition* 59: 309, 1956.
8. Putnam, P., Milam, D. F., Anderson, R. K., Darby, W. J., and Mead, P. A.: The statistical association between diet record of ascorbic acid intake and the blood content of the vitamin in surveyed populations. *Milbank Mem. Fund Quart.* 27: 355, 1949.
9. Youmans, J. B., Patton, E. W., Sutton, W. R., Kerr, R., and Steinkamp, R.: Surveys of the nutrition of populations. 5. The vitamin C nutrition of a rural population in middle Tennessee. *Am. J. Hyg.* 42: 254, 1945.
10. Storvick, C. A., Hathaway, M. L., and Nitchals, R. M.: Nutritional status of selected population groups in Oregon. II. Biochemical tests on the blood of native born and reared school children in two regions. *Milbank Mem. Fund Quart.* 29: 255, 1951.
11. Bring, S. V., Warnick, K. P., and Woods, E.: Nutritional status of school children 15 and 16 years of age in three Idaho communities; blood biochemical tests. *J. Nutrition* 57: 29, 1955.
12. Odland, L. M. and Ostle, R. J.: Clinical and biochemical studies of Montana adolescents. *J. Am. Dietet. A.* 32: 823, 1956.
13. Hard, M. M. and Esselbaugh, N. C.: Nutritional status of selected adolescent children. I. Description of subjects and dietary findings. Am. J. Clin. Nutrition 4: 261, 1956.

14. LOWRY, O. H., LOPEZ, J. H., and BESSEY, O. A.: The determination of ascorbic acid in small amounts of blood serum. *J. Biol. Chem.* 160:609, 1945.
15. KRUSE, H. D.: The gingival manifestations of avitaminosis C, with especial consideration of the detection of early changes by biomicroscopy. *Milbank Mem. Fund Quart.* 20:290, 1942.
16. Food and Nutrition Board, Nat. Res. Council (Nat. Acad. Science): *Recommended Daily Dietary Allowances*, Publication 302, Washington, 1953.
17. SNEDICOR, G. W.: *Statistical Methods*, ed. 4. Iowa State College Press, Ames, 1950.
18. STORVICK, C. A., SCHAAD, B., COFFEY, R. E., and DEARDORF, M. B.: Nutritional status of selected population groups in Oregon. I. Food habits of native born and reared school children in two regions. *Milbank Mem. Fund Quart.* 29:165, 1951.
19. WARNICK, K. P., BRING, S. V., and WOODS, E.: Nutritional status of adolescent Idaho children. I. Evaluation of seven-day dietary records. *J. Am. Dietet. A.* 31:486, 1955.
20. WILCOX, E. G. and GALLOWAY, L. S.: Children with and without rheumatic fever. I. Nutrient intake, physique, and growth. *J. Am. Dietet. A.* 303:45, 1954.
21. ODLAND, L. M., PAGE, L., and GUILD, L.: Nutrient intakes and food habits of Montana students. *J. Am. Dietet. A.* 31:1134, 1955.
22. TUCKER, R. E., CHALMERS, F. W., CHURCH, H. N., CLAYTON, M. M., FOSTER, W. D., GATES, L. O., HOGAN, G. C., STEELE, B. F., WERTZ, A. W., and YOUNG, C. M.: Cooperative nutritional status studies in the Northeast region. IV. Dietary findings. Bull. 319, Contribution 802, Rhode Island Agric. Expt. Sta., 1952.
23. BESSEY, O. A. and LOWRY, O. H.: Nutritional assay of 1,200 New York State school children. *N. Y. Legislative Documents* 7:167, 1947.
24. CLAYTON, M. M., BABCOCK, M. J., FOSTER, W. D., STREGEVSKY, S., TUCKER, R. E., WERTZ, A. W., and WILLIAMS, H. H.: Cooperative nutritional status studies in the Northeast region. V. Blood findings. Northeast Regional Publ. 14, Bull. 516, Maine Agric. Expt. Sta., 1953.
25. TISDALL, F. F. and JOLLIFFE, N.: Vitamin C malnutrition and scurvy, in *Clinical Nutrition*. Hoeber, New York, 1950, Chap. 23.
26. KRUSE, H. D.: A concept of the deficiency states. *Milbank Mem. Fund Quart.* 20:245, 1942.
27. MERROW, S. B., CLAYTON, M. M., NEWHALL, C. A., and FOSTER, W. D.: Examiners' ratings of color transparencies of clinical signs associated with vitamin deficiencies. AM. J. CLIN. NUTRITION 5:56, 1957.

The History of Pellagra, Its Recognition as a Disorder of Nutrition and Its Conquest

V. P. SYDENSTRICKER, M.D.*

THE ANCIENTS have left no leads regarding the existence of pellagra. Hippocrates, Galen, and Avicenna writing at widely separate times described no disease resembling it. Whether the condition existed among the Mexican or North American Indians is not known, though it is most likely that the early explorers would have described it. Since it is a man-made disease caused by a rather specific type of poor diet, it seems unlikely that it emerged in Spain as an epidemic about 1730 without having been present sporadically for some years. In fact it already had the popular name, *mal de rosa* when Casal first described it in 1735.

The following is a brief synopsis of the history of pellagra, omitting reference to hundreds of important contributions toward its conquest.

What does seem certain is that pellagra followed closely upon the extensive use of Indian corn for food, and this relation was noted by Casal. It was recognized in Italy in 1740 and Frapolli in 1771 attributed it to the extensive consumption of maize as "polenta," plus exposure to the sun. By 1818 it was widely prevalent in the southeastern provinces of France, and in 1830 was reported from Roumania where it had been known to exist for 16 or 18 years. It was found in Austria in 1887 and in Hungary during the following year. Egypt and Russia recognized it in the early 1890's. Here in the home of maize we do not know when pellagra appeared. Grey in Utica, New York, and Tyler in Summerville, Mass., each reported a case in 1864. The disease was diagnosed in retrospect among the Union prisoners at Andersonville but any confirmation is lacking. H. F. Harris of Atlanta in 1902 described what he considered the first authentic instance in this country. Babcock, however, reviewing the records of the South Carolina State Asylum believed that there was convincing evidence that the disease had been endemic among the inmates since 1828. Casal had emphasized the dementia of pellagra, in this country it was soon to fill the asylums of the southern states.

However high the threshold of suspicion of the American medical profession, it seems unlikely that pellagra was endemic before 1900. In 1906, Searcy reported an epidemic among the inmates of the Alabama State Asylum for Negroes, and during the ensuing year it became evident that the disease was already a major health problem throughout the Southeast. The specific reason for the explosive nature of the outbreak will never be known, though several contributing factors were probably significant. Cotton was ruinously cheap and wages correspondingly low while food prices were very high. Very few animals for food were raised, and Western meat was beyond the means of the tenant farmer and the mill worker. More important perhaps was a change in the character of the corn meal during the preceding decade. Before 1900 the bulk of the meal used in smaller towns and practically all that consumed in rural areas was locally ground in grist mills which more often than not were water-driven. "Water-ground" meal was coarsely bolted or not at all and retained much of the germ and hull of

* Professor of Medicine, Emeritus, Medical College of Georgia, Augusta, Georgia.

Dr. Sydenstricker is the recipient of the 1958 Joseph Goldberger Award in Clinical Nutrition. This award is sponsored by The Nutrition Foundation, Inc. and administered by The American Medical Association; presentation at the Annual Convention of the A.M.A. in San Francisco, June 23, 1958.

This paper was presented at the *Symposium on Problems of Human Nutrition*, co-sponsored by the Medical College of Georgia and The National Vitamin Foundation, March 4, 1958, New York, New York.

the grain. About the turn of the century, finely bolted meal produced by large milling companies made its appearance. This so-called Western meal was thoroughly degerminated to prevent the development of rancidity during storage and shipment. The meal looked better, kept better, and made more palatable bread. Also, it was much simpler for the countryman to go to the store or commissary and buy a sack of meal than to haul corn to the grist mill and return in a day or two for the ground product. In the towns, the keeping qualities of Western meal appealed to the grocers, so they stocked it exclusively. Thus, in a few years the staple cereal food of millions of people changed from what was essentially a whole grain to a rather highly refined carbohydrate that carried little to facilitate its utilization.

At the time, the general reaction of the populace including the medical profession was dismay and frustration. In the contemporary state of knowledge such an epidemic of disabling and often fatal disease could be due only to some unknown infection or subtile toxin. During the years which followed, an enormous amount of investigation was directed toward proving one or the other of those theories. Searcy's emphasis upon the mustiness of the meal used at the Alabama asylum and its heavy infection with bacteria and fungi furnished arguments for both schools of thought. Both alike overlooked the significance of the way in which the epidemic was checked. For the "poisonous" cornbread and grits Searcy substituted wheat bread and potatoes, and his patients rather promptly recovered. For the next decade old Spanish and Italian theories of the etiology of pellagra were rediscovered and had their partisans.

To compound the prevailing confusion, pellagra was recognized in the Cook County Hospital in 1909 and in the same year was found to be endemic in the Peoria State Hospital. The people of Illinois were deeply shaken, and shortly a distinguished Commission was appointed to study the disease. The report of the Commission was dogmatic: pellagra was due to some living micro-organism. In 1912, a National Pellagra Conference was held in Columbia, South Carolina. A number of outstanding foreign pellagrologists in attendance were almost unanimous in their support of the maize toxin or "mouldy meal" hypothesis. An exception was Sambon who reiterated with vigor his conviction that the disease was due to a protozoan, yet unidentified but transmitted by the bite of the buffalo gnat. This idea appealed to many American physicians and set off new investigations wherever this pest abounded.

By now pellagra had become a matter of grave national concern, it was variously estimated that the victims might number twenty-five, fifty, even a hundred thousand with deaths upward of ten thousand per year. The United States Public Health Service assigned Goldberger to an investigation of the etiology of the disease, and the Thompson-McFadden Commission undertook the same task. The Commission worked from 1912 to 1916, employing the best available methods. Extensive epidemiologic and bacteriologic studies were made and much attention was paid to sanitation. Monkeys and baboons were injected with blood, urine, cerebrospinal fluid and tissue filtrates from patients with pellagra and were fed feces and epidermal squames with entirely negative results. The Commission's report stated emphatically that infection had not been demonstrated, that no insect vector had been found, and no relation between maize and the disease had been noted. Much stress was laid on the poor sanitation of the communities investigated and the frequency of pellagra in contacts of persons with the disease. It was noted that in general the diet of the people was poor in animal protein.

Casimir Funk in 1912 suggested that pellagra might be a deficiency disease due to the lack of a "vitamine" in maize and Osborne and Mendel in 1914 demonstrated that cystine and tryptophan are essential for nutrition and growth of animals and called attention to the scarcity of those amino-acids in zein. These observations were well known to Goldberger, and he seems to have kept them in mind throughout the years, but for him it was necessary first to prove the dietary origin. During the first two years of his research, he made extensive epidemiologic studies of communities

and institutions where the disease was rife and was more than ever convinced that diet held the answer to the problem.

In a Mississippi orphanage and in the Georgia State Hospital in Milledgeville, he was able to cure the disease and prevent recurrences by adding liberal amounts of milk and eggs to the institutional diet. The next objective was to produce pellagra in previously healthy individuals by feeding them a diet similar to that of the Mississippi orphanage. The opportunity came in 1915 when a group of convicts at the Rankin Farm of the Mississippi State Penitentiary volunteered to undergo the experiment in return for pardons upon its completion. Twelve men made up the squad, and after a six-week period of observation to establish a base-line, the test was started on April 15, 1915. The diet consisted of corn meal, grits, cornstarch, wheat flour, rice, cane syrup, sugar, sweet potatoes, small amounts of turnip greens, cabbage and collards, and a liberal portion of pork fat. The average daily intake per man was protein, 41–54 g, fat, 91–134 g, carbohydrate, 387–513 g; the protein was from 80 to 97 per cent of cereal origin. One convict left the squad, but six of the remaining volunteers had developed the disease by October 31 when the experiment was terminated. In the conclusion of his report, Goldberger stated, "Pellagra may be prevented completely by a suitable diet without intervention of any other factor, hygienic or sanitary. There is no sound evidence that the disease is controllable in any other way." He also wrote, "In relation to the production of pellagra, this study suggests that the dietary factors to be considered as possibly essential are (1) an amino acid deficiency; (2) a deficient or faulty constitution of the mineral supply; possibly, but doubtfully, (3) a deficiency in the fat soluble vitamine intake; and perhaps (4) an as yet unknown (vitamine?) factor."

By this time the infectionists had rallied their forces, and there was much question whether the negative animal inoculation experiments of the Thompson-McFadden Commission could be applied to man. Goldberger and 15 courageous colleagues accepted the challenge and undertook the crucial experiment. At Spartanburg they injected themselves with blood, swabbed their throats with nasopharyngeal secretions and swallowed the excreta and squames from patients severely ill with pellagra. (Later in the experiment they put the feces, urine, and squames in capsules.) Some of the volunteers felt a bit qualmish after the dosing, but after six months no person had become ill.

During the ensuing years Goldberger sought the elusive substance lacking in a pellagra-producing diet. With Tanner he tried the addition of foods rich in all the vitamins known at that time, and failing to prevent recurrences, concluded that the deficiency was not of a vitamin but of amino acids. He reported improvement in two pellagrins whose diet was supplemented with cystine and one who received cystine and tryptophan; this was in 1922. The same year, with Wheeler, he was able to produce blacktongue in dogs with a diet essentially the same as that used in the Rankin Farm experiment and later to cure it by the addition of meat, milk, or yeast. This observation was soon applied to human pellagra at Milledgeville, and yeast produced dramatic improvement in all patients who were able to swallow and retain the 50 to 100 g per day which were required for cure.

It was now 1924 and during the nine years since the Rankin Farm experiment, the disease had maintained a steady increase throughout the South. Thanks to Goldberger's propaganda and the influence of Italian pellagrologists, dietary treatment had been almost universally adopted in hospitals so that mortality rates were lower but total deaths were not materially affected. Prevention by diet was never practical because of economic conditions as well as stubborn food habits.

Further investigation of yeast showed that the factor curative and preventive of blacktongue and pellagra resisted degrees of moist heat that entirely destroyed the beri-beri preventive factor. It was also found that the substance, now called "pellagra preventive factor" by Goldberger could be adsorbed on fuller's earth and eluted therefrom, and that the eluates were active in the cure and prevention of the disease. This led Goldberger to revert to the vitamin deficiency theory which he

had suggested some years before. Had facilities been available, the problem might have been solved at that time, since Funk had already identified nicotinic acid in yeast extracts but discarded it as a vitamin since it had no effect on beri-beri.

Voegtlin in 1914 had tried the effect of a crude liver extract with some success but abandoned the investigation when he refined the extract and found it impotent. Years later Goldberger, Wheeler, Lillie, and Rogers found dried pig's liver effective in the prevention of blacktongue. Goldberger and Rogers in 1924 tried the oral liver extract of Minot with success in the prevention of blacktongue, though Sebrell completed the experiment after Goldberger's death in 1929 and accomplished rapid cure of blacktongue with this preparation. In this year 1929 also, the incidence of pellagra reached its all time high.

On the clinical side not too much had been accomplished during the twenty-three years since the disease had been recognized. Up to 1924, hospitalized patients were fed high-protein diets, often administered by gavage and treated with a great variety of drugs of which sodium cacodylate was most commonly employed. The average mortality was 33 per cent during this era, this figure reflects the extreme delapidation of patients considered ill enough to warrant hospitalization. Complete anorexia, intractable nausea, and psychotic delusions made it impossible to administer the diet adequately. Many patients would promptly vomit after gavage, others would induçe vomiting to relieve the burning pain caused by food. Dehydration and, in retrospect, electrolyte imbalance, were the rule but at that time their importance was not generally recognized. Yeast and liver extracts for oral administration reduced the mortality rate to 25 per cent but the same difficulties that beset dietary treatment were still encountered. Injectable extracts of liver for intramuscular use proved not to be potent in the amounts used, and no crude preparation for intravenous administration had been developed. The ambulatory pellagrin seen in outpatient departments fared much better. It seldom was possible to improve the diet for any long period but those who came for help would take the yeast we gave them. Liver extracts remained too expensive to dispense to outpatients.

After Goldberger's death, Wheeler and then Sebrell headed up the research aided by many others. Up to that time the team of the U. S. Public Health Service had conducted the great bulk of the planned investigation. During the early 1930's a number of independent clinical research projects were set up throughout the country. Looking back after 30 years, one wonders how much they contributed to the actual solution of the problem but the efforts were laudable. Reported deaths exceeded 7,000 in 1928, 1929, and 1930 and there were probably more than 200,000 pellagrins in this country. The disease had truly become everybody's business.

By that time, the vitamin theory was generally accepted and whether one called the unknown vitamin "P-P factor," "vitamin G," "vitamin B_2," or just "the anti-pellagra vitamin" was unimportant. There were some even then who suspected that there might be more than one factor involved, others advanced the hypothesis of a conditioned deficiency. Riboflavin was found to be a vitamin in 1933 and pyridoxine was found to be a component of the "B group" a year later. On the basis of animal experiments in which various species of animals were used by different observers, both of these new members of the vitamin B complex were thought for a time to be the antipellagra vitamin. Much time was to pass before their true role was recognized.

Working separately, Elvehjem and his group, and Lepkovsky and Jukes with their colleagues, had found that liver extract and rice-bran extract contained a substance which was distinct from the anti-beri-beri factor of Funk (now called vitamin B_1); vitamin B_6, and riboflavin. This substance was generally known as "the filtrate factor." In 1936 Fouts, Lepkovsky, Helmer, and Jukes reported two cases of pellagra which responded to treatment with a liver preparation containing "filtrate factor" but free of vitamins B_1, B_6, and riboflavin. During the same year Sebrell, Onstott, and Hunt treated blacktongue in dogs with filtrate factor from rice bran and obtained ex-

cellent results, but in from 54 to 117 days after treatment was started, dogs developed collapse and stupor and died rapidly. Sebrell's group recognized the symptoms of "yellow liver" which they had previously described, a condition produced by certain of their deficient diets. They also noted that rats on a diet lacking riboflavin developed a similar change in the liver. Following up this observation when a surviving dog from the "filtrate factor" experiment, free from symptoms of blacktongue, developed those of "yellow liver," they treated it with riboflavin injected intramuscularly and in twelve hours recovery was complete. Sebrell then recognized that the blacktongue-producing diet was deficient both in the "antipellagra vitamin" and riboflavin and that while the "filtrate factor" contained something which cured the gastrointestinal and cutaneous manifestations of blacktongue, riboflavin deficiency developed during its administration. He also made the prophetic suggestion that it might be dangerous to treat patients with highly purified preparations of the pellagra-preventive factor, since the absence of unrecognized dietary essentials in such materials might lead to the production of illness other than pellagra.

It seems unnecessary to state, in this company, that the case was broken by Elvehjem, Madden, Strong, and Wooley early in 1937 when they identified the anti-blacktongue factor in liver extract as nicotinic acid. Their discovery was applied immediately to human pellagra wherever the disease was being studied. The results were truly dramatic. No longer did patients die because they could not retain food, yeast, or liver extracts. Nicotinic acid amide or sodium nicotinate could be given intravenously with lifesaving effect.

During the first year of nicotinic acid therapy it was noted that several patients, whose glossitis, dermatitis, diarrhea, and dementia were cured, retained certain lesions, or acquired them while being maintained on the basal pellagra-producing diet supplemented with nitocinic acid. Seborrheic dermatitis of the face, dyssebacia over the malar eminences, nose or chin, fissures of the commissures of the lips and eyelids persisted in some patients and developed in others. The tongue after the healing of the pellagrous glossitis, acquired a striking purplish-red or magenta color and the newly regenerated filiform papillae became flattened or mushroom shaped; concurrently there was scaling of the lips and redness of their buccal surfaces. Often there was complaint of burning and hyperaesthesia of the lips, tongue, and pharynx. These phenomena were at the time thought to be evidence of relapse, but in December 1938, Sebrell and Butler reported the experimental production of such lesions by a diet deficient in riboflavin with cure following administration of that vitamin. Thus Sebrell's suggestion that blacktongue and perhaps pellagra might be manifestations of deficiency of more than one essential dietary factor was justified.

While conducting further investigation of the riboflavin deficiency components of the pellagra syndrome we in Augusta became interested in superficial corneal vascularization which had been noted in riboflavin-deficient rats by Bessey and Wolbach. In this work we were privileged in having the collaboration of Sebrell and Butler and of Kruse. Our findings indicated that this lesion, though not specific, is probably the earliest sign of human ariboflavinosis.

With nicotinic acid available and quite cheap, it may seem strange that the elimination of endemic pellagra required about five years more. Yeast had been plentiful and cheap; it was furnished free by the American Red Cross and by state and county health agencies, yet the disease increased during the yeast era because the problem of prevention could not be solved. Ignorance and inertia on the part of that segment of the population which produced the pellagrins were not the whole answer. Poor food habits, economic stress, and the enormous backlog of chronic malnutrition were more important. In 1940 there were still more than 2000 reported deaths in spite of all the effort, educational and therapeutic, put forth by federal and state health organizations, the Red Cross and local welfare.

The second world war, however costly in lives and treasure, may be thanked for the conquest of pellagra in this country. The great

increase in employment and the mobilization of the armed forces provided almost everyone with an income, either from a job or a soldier's pay. The enrichment of flour put a reasonable quantity of B-vitamins back into the diet. Rationing inspired even the most backward souls to buy and eat the good high-protein foods to which they had never before aspired. Since 1945 pellagra has been a clinical curiosity seen only in the occasional food-faddist, senile recluse or chronic alcoholic. In fact, it requires considerable ingenuity at the present time to develop the disease, what with the flour and meal and grits and rice all expensively enriched with the things the mills have carefully removed.

Though pellagra ceased to be a clinical problem, the biochemical puzzle of the exact relation of diet to the disease remained unsolved. It had been observed many times that there was no direct relationship between the nicotinic-acid content of diets and their efficacy in producing blacktongue or pellagra. This suggested that there might be some other substance which could substitute for or be a precursor of nicotinic acid. The possibility of the existence of an anti-vitamin in corn was considered also. Krehl, Tepley, and Elvehjem in 1945 found that rats can synthesize niacin from tryptophan and that tryptophan supplements minimal amounts of nicotinic acid in the diet. They also showed that corn causes a marked increase in the nicotinic acid requirement. Other observers demonstrated that the same niacin-tryptophan relation exists in mice, dogs, and swine. The derivation of the vitamin from tryptophan was first reported in man by Vilter in 1949 and confirmed by Goldsmith and by Singal. More recently it has been proved that both riboflavin and pyridoxine are required for the metabolism of tryptophan and that pyridoxine is essential for the synthesis of niacin from the amino-acid.

While pellagra can develop without maize entering the picture, its prevalence in a maize-eating population can now be clarified. A diet low in good protein and containing large amounts of corn actually increases the requirement for nicotinic acid, at the same time blocking its endogenous production. If there is deficiency of riboflavin and pyridoxine as well, the utilization of what little tryptophan may be available is impossible and the diet is rendered virtually niacin-free.

At the end of the story, it is evident that many have been correct in their ideas of the genesis of the scourge. Casal, Frapolli, and the old Italian zeists were right. Funk's surmise of a vitamin deficiency and Osborne and Mendel's suggestion of amino-acid deficiency were right. Goldberger was right on three counts, diet, a vitamin, and an amino acid. Sebrell, Onstott, and Hunt were right when they suspected a multiple vitamin deficiency and Elvehjem and his group and Singal were right in pursuing the elusive tryptophan-niacin relationship.

Effect of Dietary Protein on Blood Regeneration of Anemic Patients Suffering from Parasitic Infestation

PRELIMINARY REPORT

M. DEMARCHI, M.B., CH.B.*

ANEMIA is relatively widespread in Iraq, especially among inhabitants of the rural areas where the condition results from the frequency of parasitic infestation, notably ankylostoma and bilharzia. Also, the diet of these people is deficient in certain nutritional elements, particularly animal protein, and is probably an additional factor in the causation of anemia. It was considered of interest to study the effect of various levels of protein in the diet on the regeneration of hemoglobin and red blood corpuscles, the object being to find the optimum diet in the treatment of anemia and to find out whether the administration of iron together with the irradication of parasitic infestation in a community would be all that is needed for proper treatment.

METHOD OF STUDY

The patients on whom the study was carried out were farmers or mud workers hospitalized for treatment of anemia. They were suffering from ankylostomiasis, and some of them were also suffering from bilharziasis. All the patients chosen for study were of the same socio-economic level; they were from the rural areas outside the city of Baghdad, or from the neighboring provinces. Their diet had previously consisted mainly of bread, dates, and vegetables when available. Rice was eaten two or three times weekly, and milk, meat, and eggs were occasionally consumed. On the whole, one could consider their diet to be lacking particularly in animal protein.

This preliminary report is based on the results of 53 cases studied so far. Forty-eight patients were males and five were females. The average age of the group studied was 21.68 years, with a range of 12 to 55 years. The majority of the patients were between 18 and 30 years of age. All female patients suffered from amenorrhea.

Stool examinations revealed that 51 patients were infested with ankylostoma; 16 of them were also suffering from urinary bilharzia, and three patients had ascaris as well. Two patients were suffering from bilharzia only.

The patients were divided into two major groups: the first group consisted of 27 patients who received a relatively low protein diet, and the second group consisted of 26 patients who received a modified standard hospital diet. The constituents of the two diets are shown in Tables I and II. The low-protein diet supplied 3,200 calories and 64 grams of protein; the modified standard hospital diet supplied 3,440 cal and 102.5 g protein. It was not considered practical to raise the calories in the low-

* In charge of Research, National Nutrition Institute, Baghdad.

This work has been done at the suggestion of Professor H. Gounelle, F.A.O. Nutrition Expert for Iraq.

The biochemical tests have been performed by Drs. Hasan Rabii and Mohammed Abdulnabi assisted by Leila Yousef Dhia and Nessima Abuthrab, the National Nutrition Institute Laboratories. Results will be the subject of a further publication.

The dietetic studies have been conducted by Miss Thérèse Béné, dietitian, the National Nutrition Institute of Baghdad, under the F.A.O. Technical Assistance Program. For her aid we are very grateful.

protein diet to the level of the other diet, since such a rise would have entailed the consumption of more fat and sugar. This would have rendered the diet unpalatable and at the same time intolerable, especially since it was intended that the diet be followed for one month. However there were enough calories in the low-protein diet to make available sufficient protein for tissue regeneration.

TABLE I

Daily Constituents of the Low-Protein Diet

Quantities indicated as edible part	
Breakfast	
Tea + sugar	20 g
Jam	25
Butter	20
Fruit (apple type)	100
Bread	100
Lunch	
Meat	40
Vegetables	200
Rice	150
Fruit (apple type)	250
Bread	100
Jam	25
Butter	15
4 o'clock	
Dibis*	30
Leban†	100
Tea + sugar	20
Dinner	
Salad	100
oil	10
Potatoes or	150
macaroni	60
Bread	100
Fruit	250
Jam	25
Butter	15

This diet supplies 3,200 calories and 64 g protein (8 per cent of the total calories) of which 10.7 g are animal protein.

* Date syrup

† Yoghurt

The average daily consumption of the group that received the low-protein diet was 2,900 cal and 56.4 g total protein, representing 7.8 per cent of the calories; the average daily quantity of animal protein consumed was 15 g. The group that received the modified standard hospital diet consumed an average of 3,277 cal and 103 g total protein, representing 12.6 per cent of the calories; the average daily consumption of animal protein was 37.2 g.

TABLE II

Daily Constituents of the Modified Standard Hospital Diet

Quantities indicated as edible part	
Breakfast	
Tea + sugar	20 g
Milk	150
Eggs (2) or	
"gaimar"	40
Bread	200
Lunch	
Meat	60
Rice	200
Vegetables	250
Fruit	225
Bread	100
4 o'clock	
Tea + sugar	20
Dinner	
Soup containing cereals	20
and pulses	20
Meat	50
Potatoes	150
or macaroni	60
or pulses	60
Salad	100
oil	10
Bread	100
Custard:	
milk	100
sugar	20
flour	10

This diet supplies 3,440 cal and 102.5 g protein (12 per cent of the total cal) of which 35.7 g are animal protein.

Duration of the Experiment

The majority of the patients were kept under experimental control from 26 to 32 days. In three patients, the study had to be terminated on the 21st day; in one, on the 17th day; and in another, on the 12th day.

Since there are several nutritional factors essential for hemopoiesis and in order to eliminate the most important one in the causation of ankylostoma anemia, a satisfactory supply of iron was given by mouth to all patients. Ferrous sulfate in a dosage of 2 g was administered daily. Practically all patients tolerated the drug well, and none suffered from vomiting or diarrhea. Vitamin C was not

given with the iron, nor was any liver extract or vitamin B_{12} given. However, there was some difference in the vitamin C content of both diets; the low protein diet contained more fruits and thus supplied more vitamin C than the modified standard hospital diet.

A dietitian weighed the diet of each patient and kept it on a separate tray clearly marked with the patient's bed number. This work involved the weighing of 4,548 meals for the total number of patients during the experimental period, as well as the reweighing of what was left in the dishes. During meals, patients were supervised to prevent them from offering part of their meals to other patients. All trays were checked at the end of each meal, and any foodstuff left was weighed and the amount recorded on the "ingesta" sheet for that patient.

At the end of the experimental period, calculations were made of the total consumption of each foodstuff, from which the average daily consumption was derived. The results were then expressed in terms of calories and proteins, using the International Food Composition Table (compiled by F.A.O.) or data supplied by our laboratory. Precautions were taken to prevent relatives and visitors from bringing foodstuffs to patients.

Hematologic Studies

Blood was collected from the anticubital vein, avoiding undue venous congestion. As an anticoagulant, a mixture of 0.4 per cent potassium oxalate and 0.6 per cent ammonium oxalate in the dry form was used.[1] Hemoglobin was estimated by the oxyhemoglobin method using the "Grey wedge photometer," so calibrated that the reading 100 corresponded to 14.8 g Hb/100 ml blood. Cell counts were made in duplicate, and the packed cell volume was determined by use of the Wintrobe tube centrifuged for 30 min at 3,000 revolutions per min. After being read, the tube was recentrifuged for another 15 min to ascertain that no further fall occurred in the level of the red blood cells.

The mean corpuscular volume (M.C.V.) and the mean corpuscular hemoglobin concentration (M.C.H.C.) were calculated. Tables III and IV show the results of the various hematologic studies carried out on the two groups of patients. The range of normality used for the M.C.V. was 75–96 c.μ. and for the M.C.H.C., between 28 to 34 per cent.[2] According to the classification of Wintrobe, the types of anemia in the cases studied were as noted in Table V.

TABLE III

Results of Hematologic Studies Carried Out at the Beginning of the Experiment on Patients Receiving the Low-Protein Diet

No. of patient	Hb %	R.B.C. in millions	P.C.V.	M.C.V. in c.μ.	M.C.H.C. %
1	61	3.92	33	84.2	26.8
2	28	1.97	17	86.3	24.3
3	33	2.00	14	70	34.8
4	44	4.02	32.5	80.8	19.6
5	25	1.59	16	100.6	22.6
6	60	3.73	33	88.4	26.3
7	81	3.80	38	100	30.8
8	84	4.25	43.5	102.3	28.
9	38	3.23	26	80.5	21.2
10	30	2.64	18.5	70	23.5
12	45	3.69	26	70.4	25.
13	35	2.34	17	72.6	30.4
14	43	2.64	22.5	85.2	28.2
15	36	2.62	19	72.5	28.
16	31	2.78	21	75.5	21.8
17	64	4.19	37	88.3	24.8
18	34	2.21	19	86.3	26.5
19	55	3.54	26	73.4	30.6
20	36	2.89	20.7	71.8	27.7
21	54	3.71	30	80.8	26.6
22	30	1.50	16	106.6	27.7
23	36	2.43	20	82.3	26.6
24	37	2.79	20	71.7	27.4
25	42	2.53	22	86.6	28.2
26	43	2.18	23	105.5	27.6
27	45	2.32	24	103.5	27.7
28	56	3.33	31	93.1	28.1
Mean	44.6	2.92	24.6	84.7	26.7

P.C.V. = packed cell volume; M.C.V. = mean corpuscular vol; M.C.H.C. = mean corpuscular hemoglobin concentration.

Biochemical Studies

Blood proteins, plasma iron, and the unsaturated iron binding capacity in serum were determined for most of the patients on admission as well as at the end of the study period. Analysis of these results will be the subject of a further publication.

Clinical Examination

In the group that received the low-protein

diet, one patient had an enlarged liver, three fingers' breadth below the costal margin and a spleen which reached the umbilicus. In another patient, the liver was enlarged two fingers' breadth below the costal margin. In seven patients, it was just palpable. Pitting edema was not demonstrated in any of the patients in this group, although puffiness of the face was noted in three cases. In three cases, the spleen was palpable two to three fingers' breadth below the costal margin; in four cases, it was just palpable.

In the group that received the modified standard hospital diet the liver was just enlarged in six cases. The spleen was significantly palpable in two cases, slightly in three cases, and just palpable in eight cases. There was slight pitting edema in one patient.

Body Weight: The weights of more than half of the patients (52.8 per cent) could be considered to be normal according to the "Standard Weight" table published by the Association of Life Insurance Medical Directors and Actuarial Society of America (1912).[3] The normal range was considered 10 per cent above or below the average weight. Thirty-four per cent of the patients had a weight deficit falling in the range of 10–20 per cent below average weight, and 13.2 per cent in the range of 20–30 per cent below average weight.

The greater part of the patients in both groups were dewormed at the start of the experimental period. Tetrachlorethylene in a dose of 3 ml was administered weekly for three consecutive weeks, and those patients simultaneously infested with urinary bilharziasis were also treated with fouadin. At the completion of the anti-parasitic treatment, repeated stool and urine examinations were made. In a few cases, stool examinations remained positive for ankylostoma ova even after three doses of tetrachlorethylene.

Since the dietary histories of the patients were practically the same and since the major cause of the anemia was due to intestinal blood loss caused by ankylostoma, it was postulated that the level of anemia bore some relation to the number of worms in the intestine. It was therefore decided that the blood regeneration of certain patients in both groups would be compared while they still harbored the parasites. Accordingly, two sub-groups were created for this experimental procedure as follows:

TABLE IV

Results of Hematologic Studies Carried Out at the Beginning of the Experiment on Patients Receiving the Modified Standard Hospital Diet

No. of patient	Hb %	R.B.C. in millions	P.C.V.	M.C.V. in c.μ.	M.C.H.C. %
50	35	2.59	20.5	79.1	24.7
51	44	2.90	22	75.8	29.6
52	24	2.01	14	69.6	24.8
53	40	3.20			
54	50	3.84	27	70.3	26.8
55	62	3.35	33	98.5	27.2
56	47	3.45	27.5	79.7	24.7
57	52	3.56	27	75.8	28
58	60	4.35	30	69.	29
59	50	3.62	28	77.3	25.9
60	27	1.58	15	94.3	26.6
61	40	3.04	24	78.9	25.0
62	50	3.35	26.5	79.1	28.
63	56	4.34	36.5	84.1	22.6
64	37	2.79	20.5	73.5	26.7
65	85	3.86	39	101.	32.2
66	38	2.82	21.5	76.2	26.1
67	36	2.56	22	86	24.2
68	26	1.71	14.5	84.8	26.4
69	44	3.73	29	77.7	22
70	60	3.85	31	80.5	28.6
71	49	3.15	25	79.3	29.
72	35	2.78	19	69	27.2
73	31	2.30	17	74	26.9
74	28	1.94	15	77.3	27.6
75	40	1.86	21	112.9	28.2
Mean	44	3.02	24.2	75.8	26.7

26 Patients on Modified Standard Hospital Diet		*27 Patients on Low-Protein Diet*	
Dewormed at the start	Not dewormed	Dewormed at the start	Not dewormed
13	13	20	7

RESULTS

In analyzing the results, patients had to be further classified with respect to the level of hemoglobin at the beginning of the experimental period. It has been found that the mean daily rise of hemoglobin varies with the degree of anemia at the start. Thus, a patient whose hemoglobin level at the beginning

TABLE V

Types of Anemia in Patients Receiving the Two Diet Programs

Type of anemia	Number of patients	
	Low-protein diet group	Standard diet group
Macrocytic orthochromic	2	2
Macrocytic hypochromic	4	1
Normocytic orthochromic	3	5
Normocytic hypochromic	10	11
Microcytic orthochromic	4	1
Microcytic hypochromic	4	5
Total	27	25

is 30 per cent will have a higher daily rise of hemoglobin than a patient whose hemoglobin level is 60 per cent at the start. Accordingly, patients within each sub-group were arbitrarily classified into three classes according to the level of hemoglobin: (class A) level below 40 per cent, (class B) level between 40 to 59 per cent and (class C) 60 per cent and over. Tables VI and VII show the average daily rise of hemoglobin as well as the average daily increase of red blood corpuscles in the various groups and classes.

Considering the results for the group that was simultaneously dewormed (Table VI) it will be seen that the patients whose hemoglobin level was under 40 per cent and who received the Modified Standard Hospital diet (henceforth called the MSH diet) registered an average daily rise in hemoglobin of 1.93 per cent as compared with 1.42 per cent registered by those receiving the low-protein diet. Although the number of patients is not large, the difference in rise is statistically significant. Similarly, the difference in the average daily rise in red blood cells for the two groups of the class under question is significant—a daily increase of 0.0745 million R.B.C. for patients on the MSH diet as compared with a daily average rise of 0.0487 million R.B.C. for patients on the low-protein diet.

The better effect of the MSH diet on the speed of recovery from anemia as compared with the low-protein diet is also shown in Class "B" (hemoglobin level between 40 to 59 per cent) of the same group (Table VI). An average daily hemoglobin rise of 1.86 per cent is registered on the MSH diet as compared with 1.45 per cent on the low-protein diet. This difference is also statistically significant. However, there is no difference in the average daily rise of red blood corpuscles in this class of patients.

Though the difference in the average daily rise of hemoglobin in class "C" (hemoglobin

TABLE VI

Average Daily Increase of Hemoglobin and Red Blood Cells of Anemic Patients Who Were Dewormed During Experimental Period

Type of diet	Class A Hb level below 40%			Class B Hb level between 40–59%			Class C Hb level over 60%		
	Number of patients	Hb rise %	RBC rise in millions	Number of patients	Hb rise %	RBC rise in millions	Number of patients	Hb rise %	RBC rise in millions
Modified standard hospital diet	3	1.93	0.0745	6	1.86	0.0521	4	1.31	0.0365
Low-protein diet	9	1.42	0.0487	6	1.45	0.0530	5	0.71	0.0264

TABLE VII

Average Daily Increase of Hemoglobin and Red Blood Cells of Anemic Patients Who Were Not Dewormed

Type of diet	Class A Hb level below 40%			Class B Hblevel between 40–59%		
	Number of patients	Hb rise %	RBC rise in millions	Number of patients	Hb rise %	RBC rise in millions
Modified standard hospital diet	7	1.99	0.0700	6	1.7	0.0298
Low-protein diet	4	1.34	0.0550	3	1.4	0.0300

level above 60 per cent) is marked (1.31 per cent on the MSH diet as compared with 0.71 per cent on the low-protein diet). Statistically it has not been found to be so. Similarly, no statistical significance is found in the difference between the average daily rise of red blood corpuscles in both groups.

Consideration of the group of patients who were not dewormed (Table VII) shows that the patients who fell under class "A" registered an average daily rise of hemoglobin of 1.99 per cent on the MSH diet as compared with 1.34 per cent on the low-protein diet. Statistically, the difference is very significant. The average daily rise of red blood cells for the same class of patients was 0.0700 million per cu mm of blood on the MSH diet as compared with 0.0550 million on the low-protein diet. This difference is not statistically significant. In the class "B" patients the difference in average daily rise of hemoglobin was not very significant statistically. There also was no difference in the average daily increase of red blood cells for the comparable groups.

A shift to macrocytosis following treatment with iron was found at the end of the experimental period in the blood picture of quite a number of patients. Six of the 20 patients who received the low-protein diet in the group that was dewormed at the start developed macrocytosis. Three other patients remained macrocytic. In a fourth patient, the blood picture was macrocytic at the start but changed to normocytosis. Four patients out of 11 who received the MSH diet in the corresponding group developed macrocytosis, and two already macrocytic remained unchanged.

Among the group that was not dewormed, four patients out of seven on the low-protein diet developed macrocytosis, and one already macrocytic remained so. The blood picture of eight patients out of 13 on the MSH diet also changed to macrocytosis, and one patient already macrocytic remained so.

In a previous work on the anemia of ankylostomiasis, Demarchi and Jalili[4] found similar results as regards the development of macrocytosis during treatment with iron. This phenomenon, as Lehmann[5] suggested, may be due to an outpouring of reticulocytes from a bone marrow stimulated by constant blood loss. The development of macrocytosis may be due to the existence of a dual deficiency of iron and antianemic factor; the macrocytosis of one deficiency may be masked by iron deficiency and on correction of one deficiency the other deficiency develops.[6] On the other hand, the macrocytosis may be simply the result of a macro-normoblastic reaction of the bone marrow activated by a good supply of iron in the presence of blood loss.[2,7]

CONCLUSION

Although the number of patients in each group under study is not large enough to enable one to draw a positive conclusion, there is strong indication that in severe ankylostoma anemia, a diet high in protein is more effective than a relatively low protein diet in the treatment of the anemia. The rate of recovery has been without doubt quicker on the modified standard hospital diet for the severely anemic patients. Qualitatively, there was not much difference between the two diets, since both of them contained animal protein. However, there was a difference of about 38 g in the protein content. The low-protein diet, on the other hand, was in a more favorable position as regards its vitamin C content, but it was certainly not in a similar position as regards its vitamin B_{12} content. The problem of the difference in the iron content of the two diets has been amply counteracted by the administration of a high dose of iron by mouth. Further study is in progress to find out whether a diet containing a still higher percentage of protein would give a more favorable result.

REFERENCES

1. WINTROBE, M. M.: *Clinical Haematology*, ed. 1. H. Kimpton Ltd., London, 1942.
2. JALILI, M. and DEMARCHI, M.: Classification of anaemias in Iraq. *J. Fac. Med., Baghdad* 16:71, 1952.
3. Association of Life Insurance Medical Directors and Actuarial Society of America. Medico-Actuarial Mortality Investigation, Vol. 1, p. 38, New York, 1912. Reproduced with modification in the Manual for Nutrition Surveys, prepared by the Interdepartmental Committee on Nutrition for National Defense, 1957.
4. DEMARCHI, M. and JALILI, M.: The anaemia of

ankylostomiasis in Iraq. *Al-Mihan Al-Tibbiyah* 2: 11, 1954.

5. LEHMANN, H.: Macrocytic anaemia in central Africans, in relation to ancylostomiasis and other diseases. *Lancet* 1: 90, 1949.

6. TROWELL, H. C.: Deficiency anaemias of malnutrition. *Lancet* 1: 43, 1943.

7. TROWELL, H. C.: Pernicious anaemia and macrocytic anaemia in Africans in Uganda. *Lancet* 2: 761, 1951.

Catalytic Enzyme for Medical Practice

"Today's medicine's need for an internal critique is even greater than the need of general science for self-correcting forces. Medicine is a curious mixture of arts and science. Where its practice accomplishes useful ends the arts retain their primacy and provide the medium through which science is applied to the welfare of patients. An inner critique has provided self-correcting and adjusting forces in the graphic arts, drama, and music. Politics has its correctives, be they election or revolution. But medicine has never had an effective forum for critique within and only at times fair criticism from outside. Since the conserving arts of medicine, though essential, tend to hold back progress if not examined and refreshed by the experiment and experience of practice, and since the essential characteristic of science is that it is provisional, forever being finished but never finished, *change* is the life blood of medicine. But change, unless directed, rarely correlates with progress. The crucial and painful defect of present-day medical practice is the lack of effective critical introspection. A deficiency of the catalytic enzyme, criticism, can be harmful, can produce as much morbidity and mortality in medical practice, teaching, and research as a vitamin deficiency can produce in a growing, living creature."
—W. B. Bean, *A. M. A. Archives of Internal Medicine* 97: 497, 1956.

Low Serum Vitamin B_{12} Concentrations in Alcoholics; Improvement with Liver Therapy

HUGUES GOUNELLE, M.D.* AND JEAN RICHARD, M.D.†

THE PROTECTIVE effect that vitamin B_{12} exerts on the liver seems to be well established according to reports by Burns and McKibbin, Campbell and Pruitt, Strength *et al* as well as Hove and Hardin,[1] and Popper *et al.*[2] In addition, Wolff, Royer, and Karlin[3] have determined the liver stores of vitamin B_{12} after administration of carbon tetrachloride to rats. Following administration of this toxic agent by inhalation, injection, and ingestion, a 50 per cent reduction in concentration of vitamin B_{12} in the liver was found. This reduction was related to the degree of fatty infiltration. Inasmuch as alcoholism is generally associated with various degrees of liver damage, the above findings suggested an investigation into the effects of alcoholism on the serum vitamin B_{12} concentration in humans.

We have studied both healthy individuals and 18 patients, including cases of anemia, cancer, and myeloid leukemia. The average serum vitamin B_{12} level in this group was 0.53 mμg per ml. The average total serum vitamin B_{12} level was determined after heating at 120° C for 6 minutes with KCN (pH 5.5) and in dilutions at pH 6.8[4] with *Escherichia Coli 113-3*. Further details can be obtained in work previously published.[5,6]

We also selected nine cases of chronic alcoholism hospitalized for typical gastric or neuritic disorders or for withdrawal therapy. In these subjects the low serum vitamin B_{12} concentrations were found as noted in Table I.

In these cases of chronic alcoholism the average serum vitamin B_{12} concentration was found to be 0.26 mμg/ml, or approximately one-half that of the concentration found among normal individuals.

DISCUSSION

Several factors serve to explain the 50 per cent reduction in serum levels. These may be: the low intake of nutrients with a high vitamin B_{12} content because of the lack of appetite, irregular diet, etc., and impaired absorption resulting from physiopathologic changes in the gastrointestinal mucosa.

TABLE I

Serum Vitamin B_{12} Levels in Chronic Alcoholic Patients

Patient number	Age	Clinical condition	Serum vitamin B_{12} mμg/ml
1	53	Polyneuritis, cardiac failure	0.28
2	64	Pulmonary abscess	0.35
3	69	Cor pulmonale, acute alcoholism, withdrawal treatment	0.19
4	45	Withdrawal treatment	0.24
5	29	Withdrawal treatment	0.20
6	49	Delirium tremens	0.20
7	57	Polyneuritis, senility, cardiac failure	0.40
8	51	Cirrhosis	0.22
9	43	Delirium tremens	0.28
		Average	0.26

Liver damage *per se* however is apparently not a predominant factor because in studies on five cases with extensive hepatic involvement we have found after the daily intramuscular

* Directeur du Centre de Recherches Foch, 4 Avenue de l'Observatoire, Paris.

† Docteur en Médecine.

Supported by Grant de l'Institut National d'Hygiène-Paris (Directeur Professeur L. Bugnard).

administration of liver extract* containing 10 μg of vitamin B_{12}, the average serum vitamin B_{12} concentration was within the normal range (Table II). Simple intramuscular liver therapy

TABLE II

Improvement in Serum Vitamin B_{12} Levels in Liver Disease after Intramuscular Liver Therapy*

Patient number	Age	Clinical condition and treatment	Vitamin B_{12} level *mμg/ml*
1	62	Portal hypertension and polyneuritis; 15 days of treatment for a total of 150 μg vitamin B_{12}	0.60
2	30	Cirrhosis, polyneuritis; 7 days of treatment, total 70 μg	0.54
3	45	Hepatomegaly polyneuritis; 5 days of treatment, total 50 μg	0.40
4	45	Early cirrhosis, polyneuritis; 13 days, total 130 μg	0.54
5	46	Cirrhosis, asthma; 150 μg	0.63
		Average	0.54

* Liver extract (Choay's) containing 10 μg vitamin B_{12} per 2 ml administered daily.

containing minimal amounts of cyanocobalamin produces normal values. No reason, therefore, can be found to support the frequent use of enormous doses of vitamin B_{12}, that is, from 500 to 1,000 μg. It has been reported that almost total excretion of the vitamin occurs under these conditions. The specific mechanism of the action of vitamin B_{12}, when administered intramuscularly, has not been established. As far as we know, normalization of the serum level seems to be a reasonable objective. It appears that this can be satisfactorily achieved with liver extract therapy. Jones, Mills and Capps[7] using a method with *Euglena gracilis* observed markedly elevated total serum level in patients with alcoholic cirrhosis. However, our alcoholic patients were without severe hepatic injury.

* A preparation of liver extract (Choay's) containing 10 μg vitamin B_{12} per 2 ml administered daily.

CONCLUSIONS

Serum total vitamin B_{12} concentrations were found to be low in cases of chronic alcoholism. The concentrations were 50 per cent below that of a normal group (normal 0.53 mμg per ml; the average in alcoholism was 0.26 mμg per ml).

Normalization of vitamin B_{12} concentrations was satisfactorily obtained with a daily intramuscular injection of liver extract containing 10 μg of cyanocobalamin. Mention is made of the waste that administration of large doses of vitamin B_{12} represents.

REFERENCES

1. Hove, E. L. and Hardin, J. O.: Effect of vitamins E, B_{12} and folacin on CCl_4 toxicity and protein utilization in rats. *Proc. Soc. Exper. Biol. & Med.* 77: 502, 1951.
2. Popper, H., Koch-Weser, D., and Szanto, P. B.: Protective effect of vitamin B_{12} upon hepatic injury produced by carbon tetrachloride. *Proc. Soc. Exper. Biol. & Med.* 71: 688, 1949.
3. Wolff, R., Royer, P., and Karlin, R.: Taux de la vitamine B_{12} dans le foie d'animaux intoxiques par le tetrachlorure de carbone. *C. rend. Soc. Biol.* 145: 991, 1951.
4. Wolff, R., Karlin, R., and Paysant, P.: Apropos du dosage microbiologique de la vitamine B_{12} dans le serum. *Bull. Soc. Chim. Biol.* 37: 735, 1955.
5. Richard, J.: Thèse Medecine. Paris, 1956–1957.
6. Gounelle, H. and Richard, J.: Retentissement sur la vitaminemie B_{12} de charges de cyanocobalamine administree par voie orale/rectale et parenterale. Presented March 18, 1957. To be published in *Le Sang*, 1958.
7. Jones, P. N., Mills, E. H., and Capps, R. B.: The effect of liver disease on serum vitamin B_{12} concentrations. *J. Lab. & Clin. Med.* 49: 910, 1957.

The Effect of Isonicotinic Acid Hydrazide and Vitamin B_6 on Glutamic-Oxalacetic Transaminase Levels in Whole Blood*

MARTIN SASS, M.S. AND GERALD T. MURPHY, M.D.

PYRIDOXINE or its metabolites are known to act as a prosthetic group for transaminase enzymes in animals.[1,2] While this relationship is presumably true in humans, evidence for this is indirect. Recent investigations in normal subjects[3] and in pregnant women[4] have indicated that supplementation with pyridoxine results in a significant elevation of blood transaminase activity. When supplementation is discontinued there is a slow return of blood enzyme activity to pre-administration levels.

Studies in the pyridoxine-deficient state have been less revealing. In monkeys, vitamin B_6 deficiency produced through dietary restriction resulted in a reduction of blood transaminase levels below those of control animals.[3] No such differences have been reported in humans in whom pyridoxine deficiency is not so easily established. In one study of pregnant women whose response to tryptophan loading was characteristic of pyridoxine deficiency, blood transaminase values did not differ from those of non-pregnant controls.[4] To our knowledge, no other studies have been reported on this problem.

Recent reports indicate that the administration of isonicotinic acid hydrazide (INH) in high doses can produce a syndrome in humans suggestive of pyridoxine depletion.[5] In animals, INH has been used to produce a presumably more severe pyridoxine deficiency than can be produced by dietary means or by the administration of desoxypyridoxine.[2] These observations suggested the study of blood glutamic-oxalacetic transaminase (G.O.T.) levels in patients receiving INH in routine tuberculosis therapy.

* This paper originally published in the January–February, 1958 issue, is being republished *in toto* because of some errors in the sequence of the material presented.

From the Medical Services of the Veterans Administration Hospital, Brooklyn 9, New York.

EXPERIMENT

Subjects: Six groups of patients were studied in all. Three of these (Groups A, B and C) were studied to determine whether differences in whole blood G.O.T. levels existed in individuals receiving INH as compared with individuals not receiving this drug.

Group A served as a control and was selected at random from a large group of medical and surgical patients. Selection of patients was limited by the exclusion of cases with myocardial infarction, cirrhosis of the liver or hepatitis. Group B consisted of patients from the Chest Service of this hospital, all of whom had been receiving INH (300 mg/day) for at least 30 days. Group C consisted of nine men and four women volunteers selected from laboratory and ward personnel. All of these subjects were in good health, of average income, with presumed average dietary habits. In contrast to Group A, Group C was not fed from the hospital kitchen. Two additional groups of patients (D and E) who had never before received INH were selected at random from the Chest Service. Group D was placed on INH therapy (500 mg/day) and Group E received INH (500 mg/day) plus pyridoxine (25 mg/day).

Whole blood transaminase activity was measured at the start and at suitable intervals throughout the 6 to 12 week experimental period. Complete hematologic studies were done on these patients at the start, middle and

end of the study. At the end of this experimental period, three patients in each group, D and E, received a tryptophan load test.[13]

The last group (Group F) consisted of selected patients with the lowest blood transaminase levels achieved after the administration of INH (500 mg/day) for 6 to 12 weeks. These patients continued to receive the same dose of INH plus supplementary multivitamin tablets without pyridoxine. Blood transaminase activity was measured at suitable intervals.

Laboratory Methods: Blood was collected in dry oxalate (Heller and Paul) and a 1:20 dilution was prepared in distilled water. This dilution of hemolyzed blood was centrifuged at 2500 rpm for 15 minutes to remove cell stroma. The clear supernatant was analyzed on the same day. In several cases, when necessary, the hemolysate was stored for 2 to 4 days at −20° C prior to analysis. The stability of the transaminase enzyme under these conditions is well established.

A modification[6] of the spectrophotometric procedure of Karmen and associates[7] was used for the estimation of glutamic-oxalacetic transaminase. This procedure is much more sensitive than that used by other investigators and does not present any of the reported difficulties[3,4] encountered with the use of the older procedure of Tonhazy, White and Umbreit.[8]

For this assay 0.2 to 0.3 ml of a 1:20 dilution of whole blood was analyzed. Readings were taken in a Beckman DU spectrophotometer, one unit representing a change in optical density of 0.001 O.D. units per minute, in a total volume of 3.0 ml under the conditions specified. All operations were carried out at room temperature. The temperature coefficients developed by Steinberg and Ostrow[9] were used to correct the values to a temperature of 25° C.

Lactic dehydrogenase (L.D.H.) levels were determined by an enzymatic technic based on the oxidation of reduced coenzyme I in the interconversion of pyruvic to lactic acid.[10]

The concentration of hemoglobin in the dilution of blood analyzed was determined by spectrophotometric estimation of oxyhemoglobin at 540 mμ using a Beckman DU spectrophotometer. This instrument was standardized for hemoglobin determination by the manometric procedure of Van Slyke.[11] All other hematologic data were obtained by standard technics.

RESULTS

The data in Table I indicate that the mean whole blood G.O.T. level of patients receiving 300 mg INH per day (Group B) is lower than that of the control series of hospital patients (Group A). Surprisingly enough, the mean blood transaminase level in non-hospitalized volunteers (Group C) is almost identical with that of Group B rather than with that of Group A, as might be expected.

TABLE I

Glutamic-Oxalacetic Transaminase and Lactic Dehydrogenase Activity in the Blood of Patients with and without Prolonged INH Therapy* (Mean ± S. E.)

	G.O.T.		L.D.H.
Group	Units/ml	Units/100 mg Hb	Units/mg Hb
A (Control)	723 ± 25 (23)	526 ± 20	154 ± 3.1 (12)
B (INH)*	550 ± 38 (19)	390 ± 26	152 ± 1.0 (16)
C (Volunteers)	590 ± 29 (13)	420 ± 6	157 ± 1.7 (13)
Significance (P)			
A vs. B	<0.01	<0.01	0.62
A vs. C	<0.01	<0.01	0.69
B vs. C	0.57	0.62	0.48

* INH therapy consists of 300 mg/day for a period of at least 30 days (numbers in parentheses refer to number of patients).

"P" values (Table I) indicate that the differences between Groups A and B and Groups A and C are highly significant. These differences did not change appreciably when the transaminase units were calculated on the basis of hemoglobin content indicating that variations in cell count, as reflected by hemoglobin content, did not account for the differences observed. All subsequent G.O.T. values are reported in units per 100 mg hemoglobin.

Blood lactic dehydrogenase levels (L.D.H.) of the three groups were also studied to

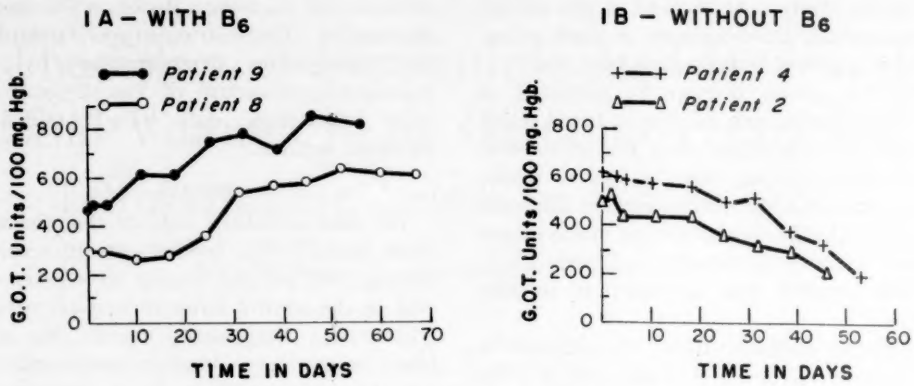


Fig. 1. Blood G.O.T. levels during INH therapy.

determine the specificity of the presumed effect of INH administration (Table I). The high "P" values indicate that there are no significant differences among the three groups.

Serial Studies: The blood G.O.T. levels during the experimental period of INH and vitamin B_6 administration are presented in Figure 1 for two representative patients in each of groups D and E. The increase in G.O.T. level during INH plus vitamin B_6 therapy (Fig. 1A) is marked beginning within 10–15 days after the start of pyridoxine administration. The decrease in G.O.T. activity observed during INH therapy alone, on the other hand, is limited and does not occur until 20–25 days of INH therapy at a level of 500 mg/day.

The transaminase values for each patient in groups D and E at the beginning and end of the experiment are presented in Table II. The differences between the mean pre- and post-therapy values for each group have been found to be statistically significant in contrast to the difference between the mean G.O.T. values for the two groups at the start of therapy. Plasma G.O.T. levels in all patients were within the normal range and could not influence whole blood transaminase levels significantly.

At the end of this experimental period three patients in each group were selected for a tryptophan load test. Twenty-four-hour urine specimens were collected on each patient for control levels. At the end of this period,

TABLE II

Change in Transaminase Activity in Whole Blood of Patients on INH Therapy with and without Vitamin B_6* (units/100 mg Hemoglobin)

	Patient	Pre-therapy	Post-therapy	Per cent change
Group D INH therapy	1	625	435	−31
	2	510	210	−59
	3	375	280	−25
	4	605	345	−43
	5	360	315	−12
	6	385	380	—
		Mean 475	330	−31
Group E INH and vitamin B_6 therapy	7	510	750	+47
	8	305	705	+130
	9	475	830	+75
	10	495	655	+32
	11	375	610	+62
		Mean 432	710	+69

* Therapy: 25 mg vitamin B_6/day and/or 500 mg INH/day over a period of from 5 to 11 weeks.

10 g DL-tryptophan* were administered by mouth and another 24-hour-urine collection was obtained. All urines were analyzed for xanthurenic acid[12] and the results are presented in Table III. They indicate that none of the patients in either group exhibited the marked increase in xanthurenic acid excretion indicative of pyridoxine deficiency.[13]

The mean hematologic values for each of the two groups at the beginning and end of the

* Generously supplied by Merck, Sharpe & Dohme, Inc.

TABLE III

Xanthurenic Acid Excretion after Tryptophan Load Test

Patient	Therapy	Xanthurenic acid mg/24 hours	
		Control*	Post-tryptophan
2	INH	29.8	48.0
3	INH	20.4	55.0
4	INH	28.8	34.4
8	INH and B_6	—	30.4
10	INH and B_6	16.4	1.9
11	INH and B_6	25.0	27.0

* 24-hour urine collection prior to administration of 10 g DL-tryptophan.

experiment are presented in Table IV. Since the hemoglobin content of the blood was used as a reference in calculating the G.O.T. level, any changes in the hemoglobin concentration per red cell (MCHC) could exaggerate changes in G.O.T. These changes did not occur. The significance of the apparent macrocytosis indicated by the elevated mean corpuscular volumes (MCV) is not apparent at the present time.

In order to evaluate the effect of vitamins other than pyridoxine, four additional patients (Group F) were studied. This group of patients had been receiving INH (500 mg/day)

TABLE IV

Hematologic Indices before and after Experimental Period

	MCV μ^3	MCH $\mu\mu g$	MCHC %	RBC *mill/mm³*	Hb *g/100 ml*	Hct %
Group D Pre-therapy	102	31.6	31.3	4.79	15.1	49
Group D Post-therapy	102	30.9	30.8	5.07	15.3	50
Group E Pre-therapy	101	29.1	28.9	4.66	13.5	47
Group E Post-therapy	100	30.2	30.4	4.70	14.2	47

MCV = mean corpuscular volume
MCH = mean corpuscular hemoglobin
MCHC = mean corpuscular hemoglobin concentration
RBC = red blood cells
Hb = hemoglobin
Hct = hemocrit

TABLE V

Transaminase Activity in the Blood of Patients on INH before and after Multivitamin Therapy* (Units/100 mg Hemoglobin)

Patient	Pre-therapy†	Post-therapy	% Change
2	210	210	0
4	280	200	−29
5	280	240	−16
6a	200	190	− 5
			Mean −12.5

* Two multivitamin tablets/day plus 500 mg INH/day for 6½ weeks.

† Mean of two determinations one week apart.

for 6 to 12 weeks and were selected on the basis of low whole blood G.O.T. levels. They were continued on the same INH therapy and were given, in addition, a daily dose of two multivitamin* tablets without pyridoxine. G.O.T. levels were measured at intervals. The results (Table V) indicate that the administration of vitamins other than B_6 did not increase blood G.O.T. levels in the dosages employed.

DISCUSSION

In animals, INH has been demonstrated to produce a pyridoxine deficiency that is more severe than can be evoked by dietary restriction or by the administration of desoxypyridoxine.[2] In humans, peripheral neuropathy has been noted after doses of INH ranging from 24 mg per kg per day to as low as 3 to 5 mg per kg per day.[14] These toxic manifestations, amenable to pyridoxine therapy, are presumably due to the formation of an INH-pyridoxine complex,[5] effectively removing pyridoxine from the available pool for tissue utilization. The relative infrequency of overt clinical signs of toxicity at lower doses of INH, however, is no assurance that more subtle biochemical changes do not occur.

Our studies indicate that the administration

* Each tablet contains the following vitamins:

Vitamin A	— 5,000 U
Vitamin D	— 450 U
Thiamine	— 2 mg
Riboflavin	— 3 mg
Nicotinamide	— 20 mg
Ascorbic acid	— 75 mg

of INH results in a drop in blood transaminase activity despite the absence of clinical signs of toxicity. The mean G.O.T. activity in the blood of patients receiving 300 mg INH per day for a period averaging approximately four months (Table I, Group B) was found to be significantly lower than that of a control group on the same diet (Table I, Group A). This dosage level constitutes the usual regimen in the therapy of tuberculosis. More recently, some investigators have concluded that this dose is inadequate therapy and have suggested the use of a minimum of 8 mg per kg per day.[15–18] At approximately this level (500 mg per day) INH has been shown to depress blood transaminase activity within a period of from 6 to 11 weeks (Fig. 1B; Table II, Group D). This effect could be reversed by the administration of 25 mg pyridoxine per day (Fig. 1A; Table II, Group E). Vitamins A and D, thiamine, riboflavin, nicotinamide and ascorbic acid were ineffective in the doses employed (Table V).

The significance of measurements of blood transaminase activity is certainly unclear at present. High doses of INH have already been shown to produce metabolic alterations indicative of pyridoxine deficiency.[5] With the dose used in this study (500 mg per day), tryptophan load tests at the end of the period of therapy were normal (Table III) in agreement with a previous report.[5] It is difficult, therefore, to avoid the suggestion that blood transaminase levels may serve as a more sensitive indicator of the adequacy or inadequacy of pyridoxine intake. The surprising finding that the mean G.O.T. activity of hospitalized control patients (Table I, Group A) exceeded that of non-hospitalized volunteers (Table I, Group C) could be a reflection of this relationship. It is certainly conceivable that the vitamin B_6 content of the routine, well-controlled hospital diet received by Group A was greater than that of the uncontrolled, non-institutional diet chosen by Group C. Unfortunately, no such measurements could be made.

Until further information becomes available on the meaning of the observed changes in transaminase activity during INH therapy, it would seem prudent to prevent their occurrence by the administration of small doses of pyridoxine whenever long-term INH therapy is necessary. The minimal dose of pyridoxine adequate to accomplish this has not as yet been determined. The amount used in this study has already been found to be effective in preventing peripheral neuritis in patients receiving 8 mg INH per kg per day.[17,18] This is probably in excess of that required to prevent changes in blood G.O.T. activity. It is expected that further study with smaller doses will indicate more precisely the amount of vitamin B_6 required to "neutralize" the effects of known amounts of INH.

SUMMARY AND CONCLUSIONS

The effect of INH and pyridoxine on whole blood glutamic-oxalacetic transaminase (G.O.T.) levels has been investigated. The mean blood G.O.T. activity of patients receiving 300 mg INH per day has been shown to be lower than that of control hospital patients but not significantly different from the mean of a group of normal, non-hospitalized subjects.

Serial studies at higher doses (500 mg INH per day) indicated that a significant drop in blood transaminase activity occurred after 6 to 11 weeks of therapy. At this time, tryptophan load tests were normal. These changes in blood transaminase activity were reversed by the administration of 25 mg pyridoxine per day and not by a group of other vitamins.

In view of these findings, it is suggested that blood G.O.T. activity may reflect the level of pyridoxine intake even before tryptophan metabolism becomes markedly abnormal. Until the actual significance of these changes becomes more clearly defined, it would seem advisable to combine the administration of pyridoxine with INH to prevent a depression in blood transaminase activity.

REFERENCES

1. Snell, E. S.: Evidence that B_6 derivatives serve as coenzymes of transaminase. *Physiol. Rev.* 33: 509, 1953.
2. Meister, A. and Downey, P. F.: Evidence for participation of vitamin B_6 in glutamine-alpha-

keto acid transamination-deamidation reaction. *Proc. Soc. Exper. Biol. & Med.* 91: 49, 1956.

3. MARSH, M. E., GREENBERG, L. D., and RHINEHART, J. F.: The relationship between B_6 investigation and transaminase activity. *J. Nutrition* 56: 115, 1955.
4. GLENDENING, M. B., COHEN, A. M., and PAGE, E. W.: Influence of B_6 on transaminase activity of human placenta, maternal and fetal blood. *Proc. Soc. Exper. Biol. & Med.* 90: 25, 1955.
5. BIEHL, J. P. and VILTER, R. W.: Effect of isoniazid on vitamin B_6 metabolism: Its possible significance in producing isoniazid neuritis. *Proc. Soc. Exper. Biol. & Med.* 85: 389, 1954.
6. STEINBERG, D. and OSTROW, B. H.: Serum transaminase as a measure of myocardial necrosis. *Proc. Soc. Exper. Biol. & Med.* 89: 31, 1955.
7. KARMEN, A., WROBLEWSKI, F., and LADUE, J. S.: Transaminase activity in human blood. *J. Clin. Investigation* 34: 126, 1955.
8. TONHAZY, N. F., WHITE, N. G., and UMBREIT, W. W.: A rapid method for the estimation of glutamic-aspartic transaminase in tissues and its application to radiation sickness. *Arch. Biochem.* 28: 36, 1950.
9. STEINBERG, D. and OSTROW, B. H.: A clinical method for the assay of serum G.O.T. *J. Lab. & Clin. Med.* 48: 144, 1956.
10. HILL, R. R. and LEVI, C.: Elevation of a serum component in neoplastic disease. *Cancer Res.* 14: 513, 1954.
11. VAN SLYKE, D. D. and NEILL, J. M.: The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. I. *J. Biol. Chem.* 61: 523, 1924.
12. ROSEN, F., LOWY, R. S., and SPRINCE, H.: A rapid assay for xanthurenic acid in urine. *Proc. Soc. Exper. Biol. & Med.* 77: 399, 1951.
13. GLAZER, H. S.: A study of urinary excretion of xanthurenic acid and other tryptophane metabolites in human beings with pyridoxine deficiency induced by desoxypyridoxine. *Arch. Biochem.* 33: 243, 1951.
14. BIEHL, J. B. and VIMITZ, H. J.: Studies on the use of a high dose of isoniazid. I. *Am. Rev. Tuberc.* 70: 430, 1954.
15. MANDEL, W., COHEN, M. L., RUSSELL, W. F., JR., and MIDDLEBROOK, G.: Serum isoniazid levels and catalase activities of tubercle bacilli from isoniazid treated patients. *Am. J. M. Sc.* 233: 66, 1957.
16. MIDDLEBROOK, G., COHEN, M. L., and SCHAEFER, W. B.: Studies on isoniazid and tubercle bacilli. III. *Am. Rev. Tuberc.* 70: 852, 1954.
17. KUKRAL, A. J., CROW, J. B., MIDDLEBROOK, G., and DRESSLER, S. H.: The handling of the poor risk patient with pulmonary tuberculosis: antimicrobial therapy, cardio-pulmonary function and surgery. *Dis. Chest* 29: 266, 1956.
18. CARLSON, H. B., ANTHONY, E. M., RUSSELL, W. F., JR., and MIDDLEBROOK, G.: Prophylaxis of isoniazid neuropathy with pyridoxine. *New England J. Med.* 255: 118, 1956.

Growth and Development of Central American Children

I. GROWTH RESPONSES OF RURAL GUATEMALAN SCHOOL CHILDREN TO DAILY ADMINISTRATION OF PENICILLIN AND AUREOMYCIN

MIGUEL A. GUZMÁN, M.S.,* NEVIN S. SCRIMSHAW, PH.D., M.D.,† AND ROBERT J. MONROE, PH.D.‡

THE MANY clear demonstrations that certain of the antibiotics increase the growth of domestic and experimental animals when added to their daily rations have been reviewed by Stokstad.[1] Studies of the effects of antibiotics upon the growth of children are relatively few and inconclusive. As early as 1951 Perrini[2] reported that the administration of 25 mg of Aureomycin® per kg of body weight per day to premature infants produced an initial two-day reduction in weight with a subsequent increase. At the end of a ten-day trial, the experimental group was 8 per cent heavier than the control group. In 1952 Snelling and Johnson[3] noted that Aureomycin lowered the morbidity, increased the growth rate and shortened the hospital stay of premature infants. Robinson[4] administered 50 mg per day of Aureomycin to twins and triplets in controlled trials, and reported that in general babies receiving the antibiotic gained more weight than the controls. Five of 15 controls died of intercurrent infections, no deaths were reported for the experimental group. MacDougall[5] has also found that administrating Aureomycin to children hospitalized with severe malnutrition resulted in more rapid recoveries and lower mortality. Her experimental group gained an average of 45.3 g daily as compared with 14.1 g for controls similarly ill.

Scrimshaw and Guzmán[6] reported that 50 mg per day of Aureomycin administered orally to Guatemalan school children for an 18-month period produced gains in both weight and height. The authors were cautious, however, in the interpretation of these results, since a true control group could not be included in the experiment. Mackay *et al.*[7] presented evidence for a slight positive effect of 50 mg Aureomycin daily to school children on weight increase but not on gain in height.

These studies are not conclusive, at least in regard to the generalization of their findings to population groups. Before attempting to draw conclusions as to the long-term effect of the continued administration of antibiotics on the growth of children, it is desirable to have more information from replicated trials with true controls. The present study has been designed for this purpose.

MATERIAL AND METHODS

The subjects were school children of predominantly Mayan origin in the 6- to 12-year

From the Institute of Nutrition of Central America and Panama (INCAP), Guatemala City, Guatemala, C. A., and the Department of Experimental Statistics, North Carolina State College, Raleigh, North Carolina.
INCAP Publication I-102.

* Chief, Division of Statistics; † Regional Advisor in Nutrition, Pan American Sanitary Bureau, Regional Office of the World Health Organization and Director, INCAP; ‡ Professor, Department of Experimental Statistics, North Carolina State College, Raleigh, North Carolina.

Assisted by grants-in-aid from Lederle Laboratories, Inc., and E. R. Squibb & Co. The data were used by the senior author in a thesis submitted to the faculty of the North Carolina State College in partial fulfillment of the requirements for the degree of Master of Science in Experimental Statistics.

age range at the start of the trial, living in two adjacent villages, San Antonio Aguas Calientes (SAAC) and Santa Catarina Barahona (SCB) in the Guatemalan mid-highlands at approximately 4,000 ft elevation. Dietary surveys carried out in each village[8,9] report the intakes of animal protein and vitamin A to be low; the consumption of other nutrients and of calories was found reasonably satisfactory. These children have been shown to be approximately two to four years behind those of the United States in weight and height, and their bone ages show a retardation of 2.5 years when compared to U. S. standards.[6]

Clinical examination revealed few of the signs usually associated with frank malnutrition excepting xerosis and follicular hyperkeratosis which were prevalent in mild degrees. No evidence of rickets or scurvy was found, and hematological studies failed to demonstrate any marked deviations from normal.[10] Serum total protein, riboflavin, vitamin A, vitamin E, and alkaline phosphatase levels in these children have been previously reported to be within normal limits, but serum vitamin A and carotene levels were found to be somewhat low.[11] In similar children in a nearby village Arroyave *et al.*[12] have demonstrated that the urinary excretion of riboflavin is very low, but that thiamine, pyridoxin and *n*-methylnicotinamide excretion are within normal limits. Parasitic infestation was heavy, espe-

TABLE I

Unadjusted Mean Rates of Growth, Age, and Treatment Frequency in Guatemalan School Children. Period One. November 1953–November 1954

Village	Sex	Treatment	No.	Weight *kg/mo*	Height *cm/mo*	Starting age	Treatment frequency
SCB*	Males	Penicillin	20	0.17	0.41	10.28	216.95
		Aureomycin	24	0.22	0.48	9.83	192.25
		Placebo	25	0.16	0.36	10.08	‡
	All males		69	0.18	0.42	10.05	203.48
	Females	Penicillin	17	0.21	0.48	9.19	206.06
		Aureomycin	23	0.22	0.47	8.53	209.70
		Placebo	25	0.20	0.44	8.80	‡
	All females		65	0.21	0.46	8.81	208.15
All SCB village			134	0.20	0.44	9.45	205.70
SAAC†	Males	Penicillin	15	0.18	0.36	9.99	203.87
		Aureomycin	26	0.23	0.44	9.42	171.04
		Placebo	33	0.17	0.39	9.90	—
	All males		74	0.19	0.40	9.75	183.05
	Females	Penicillin	12	0.25	0.43	10.24	201.33
		Aureomycin	19	0.25	0.47	9.25	164.05
		Placebo	21	0.19	0.41	9.68	‡
	All females		52	0.22	0.44	9.65	178.48
All SAAC village			126	0.21	0.42	9.71	181.08
Both villages in the period			260	0.20	0.43	9.58	193.77

* Santa Catarina Barahona.

† San Antonio Aguas Calientes.

‡ Arbitrarily defined as identically zero. Not used in computing the means. Actual frequency of placebo administration did not differ significantly from that for penicillin and Aureomycin.

cially with *Ascaris lumbricoides* and *Trichiurus trichiura*, although *Necator americanus* was not detected.[6]

All of the children in the public school in each village were divided by sex and age into three comparable groups. The same procedure was followed with the groups of new children that began school at the start of each subsequent school year.

Tablets containing either 50 mg of penicillin, 50 mg of Aureomycin or placebo (sweetened starch) were randomly assigned to the different groups of children in each village. The daily oral administration of the treatments to each individual was carefully supervised and recorded.

Weight and height measurements were taken each month during the experimental period, except during the vacation months when no measurements were taken. The treatments, however, continued to be administered in these intervals. All measurements were made during the period included between the fifth and tenth days of each month. A springless platform type scale was used for weighing the children and standard reference weights were employed for calibrating the scale at the beginning and at the end of each weighing session. The weight observations were recorded to the nearest $^1/_{10}$ pound and later converted to kilograms. For the height measurements a special wooden frame was used which permitted the direct reading of height in centimeters from a standard steel measuring tape.

Administration of the treatments started on November 5, 1953 and ended on November 10, 1955. Athough observations were made on 332 children, only 314 individuals were included in the final tabulation since all the children having three or less observations were omitted from the analysis. The observations from November 1953, through November 1954 were included in period one. The observations from December 1954, through November 1955, were included in period two. Each period, therefore, consisted approximately of a full school year and a vacation interval. Most children had enough observations to be included in both periods, although some were included in one period only. The total number of children included per treatment group, according to sex, village classification and period subdivision, are presented for period 1 in Table I and for period 2 in Table II.

The original data, which included village designation, treatment group, sex, age at the start of the experiment, monthly observations of weight and height measurements and frequency of treatment for each child, were coded and punched on IBM cards. The bulk of computations was carried out using an IBM type 650, Magnetic Drum Data Processing Machine.*

RESULTS AND DISCUSSION

The *unadjusted* means of the monthly rates of growth for period one are shown in Table I and for period two in Table II. It is apparent that the rates of increase, for both weight and height, tended to be consistently higher in period one than in period two. The examination of the individual rates of weight gain revealed that in period one there were only three children with negative rates of gain, one in the Aureomycin group and two in the placebo group while in period two there were 25 children with a negative rate of weight gain. Twelve of these children received placebos, six, penicillin and seven, Aureomycin. These observations suggest that an adverse factor of unknown nature operated during period two.

It is interesting to note that in period one, village SAAC had a slightly better rate of weight gain than village SCB while the opposite was true for the rates of increase in height. In period two, however, village SCB had the

* The analysis consisted in the computation of the simple linear regression coefficients of weight, height, log weight, and log height on the month of observation as estimates of the rates of gain for every child in each period. These rates were then analyzed for effect of age, sex, village, and frequency of treatment as well as type of supplement using a multiple regression model which included the contrasts between these factors as main effects. The study of higher order effects was carried out in four analyses of variance following the method of unweighted means. Details of the statistical techniques employed are given in the M.S. Thesis of the senior author deposited June 1956, in the library of North Carolina State College under the title "A proposed model for the evaluation of the growth responses of school children to antibiotics."

TABLE II

Unadjusted Mean Rates of Growth, Age, and Treatment Frequency in Guatemalan School Children. Period Two. December 1954–November 1955

Village	Sex	Treatment	No.	Weight *kg/mo*	Height *cm/mo*	Starting age	Treatment frequency
SCB*	Males	Penicillin	18	0.08	0.31	9.49	216.78
		Aureomycin	20	0.18	0.32	8.64	217.20
		Placebo	22	0.15	0.35	9.21	‡
	All males		60	0.14	0.33	9.10	217.00
	Females	Penicillin	17	0.24	0.39	8.53	212.53
		Aureomycin	24	0.20	0.38	8.14	227.42
		Placebo	23	0.19	0.36	7.98	‡
	All females		64	0.21	0.38	8.18	221.24
All SCB village			124	0.18	0.35	8.63	219.20
SAAC†	Males	Penicillin	12	0.16	0.31	9.95	191.92
		Aureomycin	28	0.17	0.39	8.68	176.14
		Placebo	35	0.12	0.33	8.88	‡
	All males		75	0.14	0.35	8.98	180.88
	Females	Penicillin	7	0.28	0.43	9.30	197.28
		Aureomycin	20	0.21	0.44	8.52	178.65
		Placebo	24	0.14	0.32	8.45	‡
	All females		51	0.19	0.38	8.60	183.48
All SAAC village			126	0.16	0.36	8.83	181.92
Both villages in the period			250	0.17	0.36	8.73	200.41

* Santa Catarina Barahona.

† San Antonio Aguas Calientes.

‡ Arbitrarily defined as identically zero. Not used in computing the means. Actual frequency of placebo administration did not differ significantly from that for penicillin and Aureomycin.

larger rate of gain in weight while village SAAC had the higher rate of increase in height.

In general, the rate of increase in both weight and height was faster for females in both periods. Similarly, the Aureomycin treatment apparently resulted in consistently better unadjusted growth rates than either the penicillin or the placebo treatments. Although the penicillin treatment seemed to result in somewhat increased unadjusted rates of gain in weight over the placebo group, there was enough variation in the results so that it was difficult to detect a clear trend.

The starting ages were similar for all groups, but even small differences in age proved to be important in the subsequent adjustment of the data. Due to a consistently poorer school attendance in village SAAC, there was a lower frequency of treatment in all of the groups in this village.

The unadjusted mean rates of gain for each sex and treatment in the combined locations are shown in Table III. In Table IV the same relationships are shown for the combined sexes as well as locations. The *unadjusted* means are presented for purposes of discussion but significance tests were performed only on the *adjusted* contrasts. The computed "t" values for the various contrasts are presented in Table V. The tests of significance indicate that the two villages did not differ in their rates of weight gain, but were different in their rate of increase in height ($P < 0.01$) in period one. In period two these village differences were no

TABLE III

Unadjusted Mean Rates of Growth in Guatemalan School Children According to Sex and Treatment. (Combined Locations)

Sex	Treatment	No.	Weight *kg/mo*	Height *cm/mo*	Starting age	Treatment frequency
Period One (November 1953–November 1954)						
Males	Penicillin	35	0.18	0.39	10.16	211.34
	Aureomycin	50	0.22	0.46	9.62	181.22
	Placebo	58	0.17	0.38	9.98	*
Females	Penicillin	29	0.23	0.46	9.62	204.10
	Aureomycin	42	0.23	0.47	8.86	189.05
	Placebo	46	0.20	0.43	9.20	*
Period Two (December 1954–November 1955)						
Males	Penicillin	30	0.11	0.31	9.67	206.84
	Aureomycin	48	0.17	0.36	8.66	193.25
	Placebo	57	0.14	0.34	9.01	*
Females	Penicillin	24	0.25	0.40	8.75	208.08
	Aureomycin	44	0.20	0.40	8.31	205.25
	Placebo	47	0.17	0.34	8.22	*

* Arbitrarily defined as identically zero. Actual frequency of placebo administration did not differ significantly from that for penicillin and Aureomycin.

TABLE IV

Unadjusted Mean Rates of Growth in Guatemalan School Children According to Treatment. (Combined Locations and Sexes)

Treatment	No.	Weight *kg/mo*	Height *cm/mo*	Starting age	Treatment frequency
Period One (November 1953–November 1954)					
Penicillin	64	0.20	0.42	9.92	208.06
Aureomycin	92	0.23	0.47	9.27	184.79
Placebo	104	0.18	0.40	9.64	*
Period Two (December 1954–November 1955)					
Penicillin	54	0.17	0.35	9.26	207.39
Aureomycin	92	0.19	0.38	8.49	198.99
Placebo	104	0.15	0.34	8.65	*

* Arbitrarily defined as identically zero. Actual frequency of placebo administration did not differ significantly from that for penicillin and Aureomycin.

longer significant (Table V). These observed variations between the villages may be an artifact, arising either because of different relative positions of the two villages in the alternating weight-height growth cycles, or due to chance results of sampling caused by the variation of individual observations in period one.

The rate of gain in both height and weight was significantly higher ($P < 0.01$) for the females than for the males in period one (Table V). A significant difference was also apparent in period two for the rate of gain in weight, but not in height. Different rates of gain in the two sexes were not surprising since it is well known that females usually mature sooner than males.

TABLE V

Tests of Significance of Differences in Rates of Growth in Guatemalan School Children. Computed "t" Values

Comparison	Period One (Nov. 1953–Nov. 1954)		Period Two (Dec. 1954–Nov. 1955)	
	Weight	Height	Weight	Height
Adjustment includes frequency of treatment correction				
V_2–V_1	1.20	2.99**	1.13	1.01
S_2–S_1	3.91**	4.02**	4.66**	2.24*
T_2–T_1	7.88**	9.10**	2.43*	1.17
T_3–T_1	−6.92**	−8.83**	−1.95	−1.11
T_3–T_2	8.12**	8.70**	2.65**	1.16

V_1 = SCB; V_2 = SAAC; S_1 = Males; S_2 = Females; T_1 = Penicillin; T_2 = Aureomycin; T_3 = Placebo.

* Denotes significance at the 5 per cent level.

** Denotes significance at the 1 per cent level.

Table V also shows that the responses due to Aureomycin were significantly higher than the responses due to penicillin in period one ($P < 0.01$). This is in agreement with previous findings both in animals[1,13] and in humans.[14] The results were essentially similar but the differences were less marked in the comparison of Aureomycin with penicillin in period two.

The *adjusted* treatment contrasts showed that Aureomycin significantly improved the rates of growth ($P < 0.01$), while penicillin had an inhibitory effect ($P < 0.01$) on the growth rates in period one. In period two, Aureomycin still improved ($P < 0.01$) the rate of gain in weight, but to a much lesser degree than in period one. The inhibitory effect of penicillin on the rate of gain in weight was also less marked in this period. Neither Aureomycin nor penicillin demonstrated an effect on the rate of height increase in period two.

These findings suggest that while Aureomycin results in an initial stimulus to the rates of growth, its effects eventually recede. Similarly the initial inhibitory effect of penicillin gradually lessens until it reaches its equilibrium point at a somewhat lower level than that of Aureomycin. There was, in general, considerably less uniformity in the individual responses in both weight and height to penicillin than in those to Aureomycin. The effects of statistical adjustment for village and sex differences, initial age, and treatment frequency were more marked in the children receiving penicillin than in either the Aureomycin or placebo groups.

The omission of the frequency of treatment adjustment in the analysis did not appreciably change the final interpretation of the results. The lower sensitivity in testing when this variable is omitted may, however, prove to be undesirable, especially when very small differences are involved. This sensitivity loss in testing is apparently more marked in the case of the rates of gain in weight than in the rates of height increase. Logarithmic transformations did not alter the significance of the relationships presented in Table V.

The adjusted mean rates of growth for each treatment and period are presented in Table VI. Since the experimental error estimated for period two was approximately twice as large as the experimental error estimated for period one,* the larger estimate of error for the second period was employed in carrying out all of the tests of significance in the analyses of unweighted means.

TABLE VI

Adjusted Mean Rates of Growth of Guatemalan School Children According to Treatments

Treatment	Period One (Nov. 1953–Nov. 1954)		Period Two (Dec. 1954–Nov. 1955)	
	Weight *kg/mo*	Height *cm/mo*	Weight *kg/mo*	Height *cm/mo*
Treatment frequency included in adjustment				
Penicillin	0.18	0.40	0.16	0.35
Aureomycin	0.23	0.46	0.18	0.36
Placebo	0.20	0.43	0.17	0.36
Treatment frequency omitted from adjustment				
Penicillin	0.18	0.40	0.16	0.35
Aureomycin	0.23	0.46	0.17	0.36
Placebo	0.20	0.43	0.17	0.36

No third order effect and only one second order effect proved significant; the contrast between Aureomycin and penicillin in the sexes for weight ($P < 0.01$) and for height ($P < 0.05$).

* Probably because the field personnel of the project received less close supervision of the measuring procedures in period two, an observation of interest to investigators planning similar projects.

The direction of this contrast remained the same in both sexes, but the magnitude of the observed difference for the two treatments was not the same. It may well be that when dealing with children of both sexes in the prepubertal stage of growth, any growth stimulus will result in more marked effects in the females than in the males by bringing about the onset of the pubertal growth spurt in the former.

ALTERNATIVE ANALYSIS

Prior to the more complete statistical treatment described, the results were tabulated separately by each village and by each six month period and adjusted to initial age, height, and weight by procedures previously described.[6] These data are given in Table VII and have been summarized previously in abstract form.[14] In this tabulation in which the data are uncorrected for sex difference and intensity of treatment, penicillin appears to have a significant positive effect on weight in one village and a significant negative one in the other during the first six month period, while the Aureomycin effects on height are significantly positive for both periods in these villages and for weight in neither. This illustrates the necessity of correcting not only for initial age, height, and weight variations but also for sex differences as well as for frequency of treatment. It is also clear that any experimental design which had used one village or period for a control and another village or period as an experimental group would have come to a false conclusion most of the time, yet some investigators studying treatment effects on growth in population groups have designated controls in one of these two ways. The study of the behavior of multiple experimental and placebo control groups in several time periods is essential for sound conclusions. It must be remembered that refined statistical analyses are useless unless the basic experimental design is sound and proper controls are provided.

SIGNIFICANCE OF THE RESULTS

There is little doubt that the administration of any of a number of antibiotics to children hospitalized with malnutrition results in an improvement in the rate of recovery and reduces mortality, presumably by helping the child to resist intercurrent infections. These infections particularly in an open hospital ward constitute a great hazard to the debilitated child. Whether the antibiotics have an effect on recovery beyond their influence on infection is still uncertain, but from analogy with the studies in animals, it would appear that there may also be favorable effects on their intestinal flora.

To extrapolate from this to the assumption that the continual administration of small amounts of an antibiotic orally to children living in underdeveloped areas is beneficial

TABLE VII

Adjusted Average Monthly Gain of Guatemalan School Children, May 1953 to October 1955 by Six-Month Periods

Village	Period	Weight in kg				Height in cm			
		Placebo	Penicillin	Aureomycin	Approx. L.S.D.†	Placebo	Penicillin	Aureomycin	Approx. L.S.D.†
SAAC (Groups ranged from 42–17 children)	First	0.25	0.18*	0.25	0.05	0.35	0.36	0.44*	0.05
	Second	0.18	0.17	0.18	0.05	0.51	0.51	0.51	0.05
	Third	0.18	0.19	0.26*	0.05	0.45	0.41	0.45	0.06
	Fourth	0.14	0.15	0.12	0.04	0.54	0.45*	0.54	0.06
	Fifth	0.22	0.21	0.23	0.07	0.37	0.37	0.37	0.04
SCB (Groups ranged from 39–28 children)	First	0.16	0.26*	0.22	0.06	0.43	0.51*	0.50*	0.05
	Second	0.19	0.19	0.18	0.04	0.52	0.53	0.53	0.05
	Third	0.23	0.21	0.23	0.05	0.38	0.38	0.41	0.05
	Fourth	0.15	0.19	0.16	0.04	0.51	0.54	0.55	0.05
	Fifth	0.18	0.18	0.16	0.07	0.34	0.32	0.34	0.03

* Significant differences at P = 0.05 or less.

† Least significant difference at the 5 per cent level.

would, of course, be quite unwarranted. While the results of the present study do indicate that Aureomycin, at least, does have a small but significant effect on the rate of gain in weight, this decreases with time. Aureomycin also had a slight effect on height but this had entirely disappeared in the course of the second experimental period. It is important to note, however, that even if the maximum growth effects observed had continued they would have amounted to only about 4 pounds of weight and 2 cm in height over a five year period, differences which would certainly be of little physiologic significance in the light of the many other factors influencing both weight and height. Furthermore, the data show clearly that the variations from one town to another and from one six month period to another are often far greater than the maximum effect observed from antibiotic administration.

It would be of great interest to know whether some other benefit accrued to these children, but there is no indication from vital statistics records in these towns that mortality was in any way affected and accurate morbidity data are not available. Moreover, in view of the marked retardation in the growth of these children and the sensitivity of growth to improvement in environmental factors including diet, the very slight antibiotic effects on growth observed do not make it be probable that there were other collateral benefits of significance. This is partially confirmed by the failure of antibiotic supplements in previous experiments by the authors to influence either hematological values[10] or the levels of a number of serum constituents.[11]

For all of these reasons we conclude that despite the obvious value of antibiotics in clinical medicine there is no present justification for considering their continued administration to children in underdeveloped areas to be an effective or desirable public health measure. The present findings and conclusions agree with those of the concurrent study of Mackay *et al.*[7] in Jamaica.

SUMMARY AND CONCLUSIONS

Groups of children aged 6–12 years of predominant Mayan Indian origin from each of two schools in adjacent rural communities in the Guatemalan highlands received daily doses of 50 mg of either penicillin or Aureomycin for a period of 25 months. A third group in each school received placebo. All of these school children subsisted on marginal diets low in animal protein. Age at the start of the experimental period, monthly weight and height observations, as well as a record of treatment frequency constituted the basic data.

For the purposes of analysis, the over-all experimental period was subdivided into two shorter periods of approximately equal duration. Regression methods were used to estimate the rate of growth of each child, and the computed regression coefficients served as the variable for analysis. The results indicated an initial stimulatory effect of Aureomycin on the rates of growth on both weight and height. The effect on the rate of gain in weight was greater than that on the rate of gain in height, and decreased markedly with time. On the other hand, penicillin seemed to have an inhibitory effect on both rates of gain, which became insignificant with time. The treatment effects were greater in females than in males. In some cases the comparative effect of penicillin and Aureomycin changed with the sex. In general, the growth rates for period two were lower than the growth rates for period one.

Additional information from an alternative analysis illustrates the necessity of correcting not only for initial age, height, and weight but also for sex differences as well as for frequency of treatment. It is also evident that the study of the behavior of multiple experimental and placebo control groups in several time periods is essential for sound conclusions.

REFERENCES

1. Stokstad, E. L. R.: Antibiotics in animal nutrition. *Physiol. Rev.* 34:25, 1954.
2. Perrini, F.: Aureomycin as a growth factor in premature infants. *Boll. Soc. Ital. Biol. Sper.* 27: 1151, 1951.
3. Snelling, C. E. and Johnson, R.: The value of aureomycin in prevention of cross infection in the hospital for sick children. *Canad. M. A. J.* 66:6, 1952.
4. Robinson, P.: Controlled trial of aureomycin in premature twins and triplets. *Lancet* 1:52, 1952.

5. MacDougall, L. G.: The effect of aureomycin in undernourished African children. *J. Trop. Pediat.* 3: 74, 1957.
6. Scrimshaw, N. S. and Guzmán, M.: The effect of dietary supplementation of vitamin B_{12} and aureomycin on the growth of school children. *Current Research of Vitamins in Trophology*, The National Vitamin Foundation, Nutrition Symposium Series No. 7, 1953, pp. 101–117.
7. Mackay, I. F. S., Patrick, S. J., and Stafford, D.: The influence of vitamin B_{12} and aureomycin upon the growth of protein-deficient children. *J. Nutrition* 59: 155, 1956.
8. Flores, M. and Reh, E.: Estudios de hábitos dietéticos en poblaciones de Guatemala, III. San Antonio Aguas Calientes y su aldea, San Andrés Ceballos. Suplemento No. 2, *Boletín de la Oficina Sanitaria Panamericana*, Publicaciones Científicas del INCAP, 1955, pp. 149–162.
9. Flores, M., Flores, Z., and Meneses, B.: Estudios de hábitos dietéticos en poblaciones de Guatemala. IX. Santa Catarina Barahona. *Arch. Venezolanos de Nutrición*, 1957, in press.
10. Aguirre, F., Scrimshaw, N. S., Muñoz, J. A., and Cabezas, A.: The effect of supplements of animal and vegetable protein, vitamin B_{12} and aureomycin on hematological values in Central American school children. Am. J. Clin. Nutrition 3: 225, 1955.
11. Guzmán, M. A. and Scrimshaw, N. S.: Serum ascorbic acid, vitamin A, carotene, vitamin E, riboflavin and alkaline phosphatase values in Central American school children. *Fed. Proc.* 11: 445, 1952.
12. Arroyave, G., Sandstead, H., and Schumacher, R.: Relation of urinary creatinine and vitamins to nutritional status in pre-school children. *Fed. Proc.* 17: 469, 1958.
13. Braude, R., Wallace, H. D., and Cunha, T. J.: The value of antibiotics in the nutrition of swine: A review. *Antibiotics & Chemotherapy* 3: 271, 1953.
14. Scrimshaw, N. S., Guzmán, M. A., and Tandon, O. B.: Effect of aureomycin and penicillin on the growth of Guatemalan school children. *Fed. Proc.* 13: 477, 1954.

Diet Therapy

The Amino Acid Requirements of Adults

A REVIEW

MAY S. REYNOLDS, PH.D.*

THE TERM protein, used to describe the nitrogenous substance found in body tissue, was coined from the Greek verb meaning "to take first place" and gives some indication of the importance that was early attached to that fraction of the diet.

It soon became evident that proteins differ in their value and that such differences are due to amino acid composition. The early protein studies of Osborne and Mendel and of other investigators have been thoroughly reviewed by Rose.[1] A little more than 20 years ago, Rose[2] reported on the qualitative and quantitative amino acid requirements for normal growth of the rat. The next step was to find that of the ten amino acids essential in the ration of the growing rat, eight must be furnished in the diet to maintain nitrogen balance in adult man. Finally, the amount of each that is required for that function of nitrogenous compounds was determined. The results of these laborious and extensive studies were first summarized in a publication in 1949.[3] More recent studies of the requirements of women for the essential amino acids have been reported by Leverton and co-workers[4–8] Swendseid and Dunn[9,10] and by workers in the laboratories of the University of Wisconsin.[11,12] The quantitative requirements of adult men and women as found in the foregoing studies are given in Table I.

The general plan of all of these studies using human subjects was much the same. Subjects were fed a carefully controlled semisynthetic diet which provided generous amounts of the essential amino acids with additional nitrogen for the synthesis of the dispensable amino acids. Nitrogen balance was the criterion of adequacy. When the subjects had attained nitrogen balance on the "complete" amino acid mixture, the amino acid for which the requirement was being determined, was removed to produce a negative nitrogen balance and then replaced stepwise until balance resulted. Calories were provided in amounts sufficient to just avoid weight gain. It is of interest to note that as the subjects were changed from a diet of ordinary foods to the semisynthetic diet, it was neces-

TABLE I

Minimum Amounts of Essential Amino Acids Required for Maintenance of Nitrogen Balance in Adults

Essential amino acid	Women g/day	Men
Isoleucine	0.450	0.70
Leucine	0.620	1.10
Lysine	0.500	0.80
Phenylalanine with tyrosine present	0.220 0.900	1.10 —
Sulfur-containing amino acids	0.550	—
Methionine	0.290	1.10
Cystine	0.250	—
Threonine	0.310	0.50
Tryptophan	0.160	0.25
Valine	0.650	0.80

From: REYNOLDS, M. S.: *J. Am. Dietet. A.* 33:1016, 1957.

* Professor, Departments of Food and Nutrition, School of Home Economics, University of Wisconsin, Madison.

sary in most cases to increase calories. In our own studies, an increase in calories of from 13 to 33 per cent or an average of 18 per cent was needed in order to attain nitrogen balance and prevent weight loss. Rose[13] has reported a similar increase in caloric requirement.

The amino acid requirements of women appear to be somewhat less than those of men as reported by Rose. However, Clark *et al.*[14] observed no striking difference in the quantities of lysine required by men and women for nitrogen equilibrium. The latter investigators report a statistically significant relationship of lysine requirement to body weight, surface area, creatinine excretion and metabolic size. These findings differ from those of Rose *et al.*[13] and of Jones, *et al.*[11] who found no relation of requirement to body size. The requirement for lysine as reported by Clark *et al.*[14] is greater than that reported by Rose[3] for men or by Jones *et al.*[11] for women.

These requirements are basic to further research in this area. They represent laborious effort on the part of the investigators and real self-sacrifice by the subjects. In order that these results may be most valuable, it is important to recognize their limitations as well as their contributions. These determinations have been made on a small number of subjects, under carefully controlled conditions and for relatively short periods of time. Length of time on the experimental diet may be a factor that needs consideration. For example, it has been suggested by Nassett[15] that histidine may not actually be a dispensable amino acid over a long period of time, but for a short period may be furnished by the degradation of hemoglobin.

The proposed requirements represent only the quantities needed to maintain nitrogen balance. There is no doubt of greater requirements for some other functions of amino acids. For example, Chaloupka, and co-workers[16] working with rats and Vivian, and associates[17] with human subjects, found that a lower intake of tryptophan was needed to maintain nitrogen balance than to form blood pyridine nucleotides. Greater differences probably exist in the requirements for growth, reproduction, antibody formation, and for the many other functions of amino acids in the body. Total calories, total nitrogen, previous diet, distribution of amino acids, and many other factors undoubtedly influence the requirements.

The limitations as well as the contributions of these studies of amino acid requirements have been discussed in a previous paper.[18] Recognition of the limitations of available data is not intended to underestimate their value. They represent basic and fundamental information which, if properly applied, can contribute to the solution of many problems of protein nutrition.

Available data on the amino acid composition of self-selected diets[20] in this country and on the amino acid content of the "Average American Diet"[19] indicate that problems of protein deficiency are not directly the result of an intake of the essential amino acids below the proposed requirements (Tables II and III).

TABLE II

Amino Acid Intakes of Women on Self-chosen Diets

Essential amino acid	Intake g/day		
	Women in nitrogen balance	Women in negative nitrogen balance	Minimum requirements of women for essential amino acids
Isoleucine	1.62–5.73	1.15–4.88	0.450
Leucine	4.83–7.35	3.37–6.25	0.620
Lysine	3.25–7.17	1.70–5.85	0.400
Methionine	0.83–2.54	0.67–2.03	0.290
Cystine	—	—	(0.250)
Phenylalanine	2.88	1.53–4.03	0.220
Tyrosine	—	—	(0.900)
Threonine	1.44–3.44	0.97–3.02	0.310
Tryptophan	0.44–1.28	0.25–0.91	0.160
Valine	3.20–4.58	2.51–5.98	0.650
Total nitrogen	8.20–15.20	4.50–14.40	10.000

From: Reynolds, M. S.: *J. Am. Dietet. A.* 33:1016. 1957.

We must keep in mind, however, the fact that the experimental diets furnished 6 to 10 g nitrogen/day as well as a generous intake of calories. A diet low in protein and possibly also low in total calories may easily meet the requirements for essential amino acids or even the Rose "safe levels" and still not furnish adequate total protein, through failure to furnish enough

TABLE III

Essential Amino Acid Content of the "Average" American Diet Compared with the "Safe" Levels for Men

Essential amino acid	"Average American" diet	"Safe" levels for men
	g/day	
Isoleucine	4.2	1.40
Leucine	6.5	2.20
Lysine	4.0	1.60
Methionine	3.0	2.20
Phenylalanine	4.1	2.20
Threonine	2.8	1.00
Tryptophan	0.9	0.50
Valine	4.2	1.60

From: REYNOLDS, M. S.: *J. Am. Dietet. A.* 33: 1016, 1957.

nitrogen for the synthesis of the so-called dispensable amino acids.

These data on amino acid requirements have provided a basis for describing a pattern for a provisional reference protein.[21] Comparison of the amino acid composition of a protein with that of the reference protein has been proposed as a means of determining the biologic value of the protein and has been found to agree quite closely with biologic determinations. It is quite possible, however, that in some foods, the concentration of amino acids made available to the body as a result of *in vivo* digestion may not be the same as that found by analysis following drastic methods of hydrolysis. Biologic trials may be necessary to verify values based on calculations.

As the commercial preparation of certain amino acids at a low cost has become practical, the possibility of supplementing cereal proteins with amino acids to balance their deficiencies immediately suggests itself. Flodin[22,23] has presented data showing how all common cereal proteins can be improved in this way. This proposal has some merit for a diet in which a large proportion of the total protein is furnished by cereals. The fortification of wheat proteins with lysine represents a practical application of the principle. However, the value or necessity of such supplementation in bread containing milk proteins, as included in the "Average American diet" remains to be demonstrated. By reference to Tables II and III it may be noted that the self-selected diets of women and the "average American diet" furnish lysine in amounts from two to three times the Rose "safe" levels for men. Moreover, such a procedure should be approached with caution and should not be looked upon as opening the way for indiscriminate amino acid supplementation. Scrimshaw and associates[24] reported on the effects on nitrogen retention of supplementing a corn preparation with lysine, tryptophan, and methionine. Nitrogen retention improved with the addition of lysine and tryptophan but decreased when methionine was added. He suggested that the added methionine may have created some type of imbalance. "Whether or not the supplementation of diets with synthetic amino acids will prove practical and desirable can only be determined by fundamental studies to obtain data not now available."[24] The effects of imbalances of amino acids that may be created when availability of amino acids is not known have been reported by Elvehjem.[25]

A statement on the "Supplementation of Dietary Proteins with Amino Acids" adopted in October 1956, by the Food and Nutrition Board of the National Research Council[26] says: "The imbalance of essential amino acids found in some dietary proteins cannot always be corrected by adding a single amino acid, the imbalance being the result of a deviation in several of the essential amino acids from an 'ideal pattern' needed by the body. Multiple supplementation is generally required. This type of supplementation is at present best achieved by mixed diets where one food protein supplements another. The benefits to be derived from amino acid supplementation are uncertain until our knowledge of the consequences of amino acid imbalance is more complete. The Food and Nutrition Board recognizes the potential value of proper supplementation with amino acids and the desirability of intensive study of this problem."

In conclusion, data on amino acid requirements represent basic information, upon which a wide range of additional studies can be based. The need is apparent for studies of the effect of other factors in the diet on amino acid requirements and for data on the requirements of

additional age groups. Information concerning the utilization of amino acids of foods is imperative before widespread use can be made of current findings. These investigations are laborious and expensive but should be undertaken as a contribution to solving the protein problems of the world.

REFERENCES

1. ROSE, W. C.: Nutritive significance of amino acids. *Physiol. Rev.* 18: 109, 1938.
2. ROSE, W. C.: The nutritive significance of the amino acids and certain related compounds. *Science* 86: 298, 1937.
3. ROSE, W. C.: Amino acid requirements of man. *Fed. Proc.* 8: 546, 1949.
4. LEVERTON, R. M., GRAM, M. R., CHALOUPKA, M., BRODOVSKY, E., and MITCHELL, A.: The quantitative amino acid requirements of young women. 1. Threonine. *J. Nutrition* 58: 59, 1956.
5. LEVERTON, R. M., GRAM, M. R., BRODOVSKY, E., CHALOUPKA, M., MITCHELL, A., and JOHNSON, N.: The quantitative amino acid requirements of young women. 2. Valine. *J. Nutrition* 58: 83, 1956.
6. LEVERTON, R. M., JOHNSON, N., PAZUR, J., and ELLISON, J.: The quantitative amino acid requirements of young women. 3. Tryptophan. *J. Nutrition* 58: 219, 1956.
7. LEVERTON, R. M., JOHNSON, N., ELLISON, J., GESCHWENDER, D., and SCHMIDT, F.: The quantitative amino acid requirements of young women. 4. Phenylalanine, with and without tyrosine. *J. Nutrition* 58: 341, 1956.
8. LEVERTON, R. M., ELLISON, J., JOHNSON, N., PAZUR, J., SCHMIDT, F., and GESCHWENDER, D.: The quantitative amino acid requirements of young women. 5. Leucine. *J. Nutrition* 58: 355, 1956.
9. SWENDSEID, M. E., WILLIAMS, I., and DUNN, M. S.: Amino acid requirements of young women based on nitrogen balance data. 1. The sulfur-containing amino acids. *J. Nutrition* 58: 495, 1956.
10. SWENDSEID, M. E. and DUNN, M. S.: Amino acid requirements of young women based on nitrogen balance data. 2. Studies on isoleucine and on minimum amounts of the eight essential amino acids fed simultaneously. *J. Nutrition* 58: 507, 1956.
11. JONES, E. M., BAUMANN, C. A., and REYNOLDS, M. S.: Nitrogen balances of women maintained on various levels of lysine. *J. Nutrition* 60: 549, 1956.
12. REYNOLDS, M. S., STEEL, D. L., JONES, E. M., and BAUMANN, C. A.: Nitrogen balances of women maintained on various levels of methionine and cystine. *J. Nutrition* 64: 99, 1958.
13. ROSE, W. C., COON, M. J., and LAMBERT, G. F.: The amino acid requirements of man. 6. The role of the caloric intake. *J. Biol. Chem.* 210: 331, 1954.
14. CLARK, H. E., MERTZ, E. T., KWONG, E. H., HOWE, J. M., and DELONG, D. C.: Amino acid requirements of men and women. 1. Lysine. *J. Nutrition* 62: 71, 1957.
15. NASSETT, E. S. and GATEWOOD, U. H.: Nitrogen balance and hemoglobin of adult rats fed amino acid diets low in L- and D-histidine. *J. Nutrition* 53: 163, 1954.
16. CHALOUPKA, M. M., WILLIAMS, J. N., JR., REYNOLDS, M. S., and ELVEHJEM, C. A.: Relative roles of niacin and tryptophan in maintaining blood pyridine nucleotides, nitrogen balance and growth in adult rats. *J. Nutrition* 63: 361, 1957.
17. VIVIAN, V. M., CHALOUPKA, M. M., and REYNOLDS, M. S.: Some aspects of tryptophan metabolism on low niacin intake. *Fed. Proc.* 16: 401, 1957.
18 REYNOLDS, M. S.: Amino acid requirements of adults. *J. Am. Dietet. A.* 33: 1015, 1957.
19. BLOCK, R. J. and BOLLING, D.: *The Amino Acid Composition of Proteins and Foods*, ed. 2. Thomas, Springfield, Ill., 1951.
20. REYNOLDS, M. S., FUTRELL, M. F., and BAUMANN, C. A.: Nitrogen balances and amino acid content of self-selected diets of women. *J. Am. Dietet. A.* 29: 359, 1953.
21. ALLISON, J. B.: Evaluation of dietary proteins. *Nutrition Rev.* 14: 129, 1956.
22. FLODIN, N. W.: The philosophy of amino acid fortification of foods. *Cereal Science Today* 1: 165, 1956.
23. FLODIN, N. W.: Amino acids and proteins. Their place in human nutrition problems. *J. Agr. Food Chem.* 1: 222, 1953.
24. SCRIMSHAW, N. S., SQUIBB, R. L., BRESSANI, R., BÉHAR, M., VITERI, F., and ARROYAVE, G.: Vegetable protein mixtures for the feeding of infants and young children; in *Amino Acid Malnutrition, XIII Annual Protein Conference*, Rutgers Univ. Press., New Brunswick, N. J., 1957, pp. 28–46.
25. ELVEHJEM, C. A.: Amino acid balance in nutrition. *J. Am. Dietet. A.* 32: 305, 1956.
26. Committee on Amino Acids: Statement on supplementation of dietary proteins with amino acids. *Proc. Food & Nutrition Bd.* 16: 41, 1956.

Diet Lists

FROM TIME TO TIME we will publish diet lists which seem to have clinical usefulness. Minor variations exist in all standard diet lists; nevertheless these are representative and may be considered useful guides. Publication does not necessarily imply complete agreement with all details.

The following diet list may be obtained from J. B. Roerig and Company, 800 Second Avenue, New York 17, N. Y.

Reducing Diet

Diet for _____

Your total calories should not exceed_____for each day. Divide them as directed below. *Do not eat between meals.*

Breakfast:

You may select from below____ calories.

	Calories
4 oz. corn flakes	48
6 oz. glass milk	135
6 oz. glass skimmed milk	68
1 teaspoon sugar	20
1 egg	75
Tea or black coffee, no sugar	0
Saccharine, 1/4 grain tablet	0
1/2 medium orange	26
1/2 medium grapefruit, 4″ diameter	20
1 teaspoon butter	36
1/2 cup oatmeal, cooked	74
1 slice rye or white bread	60
4 oz. glass tomato juice	25
1 small or 1/2 medium banana	45
1 plain soda cracker	24

Lunch:

You may select from below____ calories.

	Calories
1/2 cup asparagus tips (10 stalks)	16
5 Brussels sprouts	12
3 stalks fresh celery	9
10 slices cucumbers	15
1/3 small head of lettuce	12
1/2 cup cooked spinach	12
1/2 cup canned tuna fish	200
1/2 cup canned salmon	200
3 tablespoonfuls cottage cheese	55
1 egg	75
1 slice rye or white bread	60
2/3 cup cooked cabbage	20
1/2 cup eggplant	14
1/2 cup string beans	10
1 medium fresh tomato	20
1/3 cup carrots	18
1/3 cup turnips	13
1 small apple, raw	56
1/4 muskmelon	24
Tea or black coffee, no sugar	0
Saccharine, 1/4 grain tablet	0
1/2 cup broccoli	21
2/3 cup cauliflower	12
1/3 cup beets	32
1/4 cup white onions (2–3 small)	24
1/3 cup green peas, fresh or canned	34

Dinner:

You may select from below____ calories.

	Calories
8 oz. chicken soup	75
1 cup tomato soup	90
1 cup beef broth	100
2 slices lean roast beef, 2″x3″x 1″	179
3 oz. hamburger steak	316
3 oz. lean round steak	140
2 slices lean roast lamb, 1″x4″x 1″	211
1 slice rye or white bread	60
2 slices roast veal, 2″x3″x1/2″	152
1 slice breast chicken	100
1/2 chicken broiler, medium	120
1 egg	75
1 fillet haddock, 4″x3″x1/2″	158
5 large oysters	60
Tea or black coffee, no sugar	0
Saccharine, 1/4 grain tablet	0
2 slices halibut, 4″x2″x1/2″	126
1/2 cup shrimps	85
1 frankfurter	124

Bread, beverages, fruit, etc., from the above list, may be added to any meal provided the total calories do not exceed the amount prescribed. Six glasses of liquid each day are allowed. Be sure that your daily menus include a fresh fruit, either meat, fish or egg, milk, and three vegetables.

Special Instructions: _____

_____

Next Appointment: _____

The following high calorie foods must be avoided.

Nuts
Olives
Olive oil
Chocolate and cocoa
Gravy
Cream soups
Sauces
Ice cream
Sweet or sour cream
Candy
Pastry
Macaroni
Potatoes

Alcoholic beverages
Canned fruits in syrup
Highly spiced or salted foods

Learn to count the number of calories in the foods you eat.

Here are some examples of foods which contain many calories:

	Calories
Candy bars (per bar)	200–300
Pie (1 serving)	300–475
Cake (1 serving)	200–475
Ice cream (1 serving)	200–250
Sundaes	350–450
Ice cream sodas	325–400
Malted milk shakes	450–600
Sandwiches	200–450
Beer (8-oz. glass)	110
Cocktails or highballs	150–300
Nuts (small serving—10)	100

Do not use sugar unless you absolutely have to. No mayonnaise on salads. (Lemon juice may be used instead.)

Reviews of Recent Books

The Pharmacologic Principles of Medical Practice, fourth edition, by J. C. Krantz, Jr. and C. J. Carr, Williams and Wilkins, Baltimore, 1958, pp. 1,313, $14.00.

Pharmacology is currently undergoing an unparalleled expansion. The vast numbers of new drugs, new types of therapeutic substances, and new concepts of drug action make this a most rapidly changing branch of medicine. Textbooks of pharmacology, therefore, are always in danger of being "out-of-date." The fourth edition of this well known text is remarkable for at least one reason; it contains adequate descriptions of many of the most recently introduced compounds. This would seem to be an important advantage. Another useful technic is the free use of common and trade names wherever a drug is more familiarly known by it.

The fourth edition contains many new drugs while others, no longer generally used, have been deleted. A number of full-page photographs of eminent workers in the field add to the general interest of the book. Good printing and a full index should be noted. This edition can be recommended as an exceptionally current standard text in clinical pharmacology. S. O. W.

Clinical Gastroenterology by Eddy D. Palmer, Paul H. Hoeber, Inc., New York, 1957, pp. 630, $18.50.

This attractive book contains, in addition to chapters on diseases of the various portions of the gastrointestinal tract and its appendages, a short chapter on the spleen and one on gastrointestinal manifestations of certain far-removed diseases. Parasites are also discussed.

The introductory chapter is devoted to comprehensive gastroenterology in which the functional and emotional aspects of gastrointestinal disease in general are emphasized. In subsequent sections dealing with specific diseases, the psychosomatic and functional aspects of certain gastrointestinal diseases such as ulcerative colitis, peptic ulcer and irritable colon are very well covered; those dealing with achalasia, chronic gastritis, and regional enteritis, less so. No space is devoted to the psychosomatic aspects of nontropical and tropical sprue.

The book is extremely well written, readable, and contains much data which should be of value to all who deal with gastrointestinal problems. The author draws freely on a rich clinical personal experience and expresses personal opinions on controversial subjects. A brief list of pertinent references is included at the end of each chapter. The 216 figures are excellent and informative; they consist of descriptive sketches, photographs of specimens, and reproductions of roentgenograms.

The book is highly recommended for general practitioners, internists, specialists in gastroenterology, and surgeons. It is also recommended to medical students as an excellent source of modern information on the clinical aspects of gastrointestinal diseases.

T. E. MACHELLA

Diabetes as a Way of Life, by T. S. Danowski. Coward-McCann, Inc., New York, 1957, pp. 177, $3.50.

This guide for diabetic patients contains much practical information for their self-care. The material is presented in an attractive and readily comprehensible style. The contents cover the important details of dietary management utilizing the food exchange system and provide a thorough discussion of insulin therapy. The methods employed by the author are not always those recommended in other clinics but nevertheless are widely used in the care of diabetics. For example, the sterilization of the syringe by boiling rather than by alcohol or Zepharin is not always advised; under the section on surgery, the reversion to regular insulin during operation advocated by the author is not practised in all centers. The final chapter dealing with the life of the diabetic and the impact upon the individual and his family should be read by all those involved with this disease. A glossary of terms for the diabetic patient and a useful appendix containing detailed material on various subjects conclude this unique and informative manual. C. R. SHUMAN

Biochemistry and Human Metabolism, by Burnham S. Walker, William C. Boyd, and Isaac Asimov. Williams and Wilkins, Baltimore, 1957, pp. 895, $12.00.

This is the third edition of an excellent biochemistry textbook first published in 1952. Since the first edition was reviewed in this JOURNAL (1: 405, 1953), only the revisions will be discussed here. Large parts of the book have been rewritten, enlarged, and reorganized to bring the subject matter up-to-date and to make it clearer to the student. This is particularly true of the chapters on protein metabolism, heat and work, and infection. The bibliographies after each chapter and the index at the end of the book have been greatly enlarged helping to make the book valuable as a reference

text. New material has been introduced, such as the carbon cycle in photosynthesis, high-energy acyl-mercaptan bond, aldosterone, lipocaic acid, chemotherapy, abnormal hemoglobin, and function of metallo-flavoenzymes.

In the older edition the appendix contained chapters on colloids, isotopes, thermodynamics, and acid and base. These subjects, with the exception of the isotope section have now been incorporated into the main body of the book. Since this material is not used to any great extent in the biochemical discussion, it might have been better left in the appendix. However, this is but a minor criticism of a book which would make a valuable addition to the library of anyone interested in the clinical aspects of biochemistry.

The authors are to be complimented for keeping this book so well up-to-date. MARGARET W. BATES

Technique of Fluid Balance. Principles and Management of Water and Electrolyte Therapy, by G. H. Tovey. Thomas, Springfield, 1957, pp. 100, $2.50.

The significance of fluid and electrolyte balance in clinical medicine has become fully appreciated by practitioners as it has been by investigators for some years. The wide interest in this important phase of treatment is apparent when one considers the number of texts recently published dealing with the subject. While an understanding of the fundamental principles of biochemistry and physiology with respect to fluid balance is required for success in management, it is possible to provide the necessary details in a simple form easily applied to both elementary and complex problems. This small volume presents a succinct, yet adequate, discussion of the basic concepts of water and electrolyte metabolism. The classic description of salt and fluid depletion states are given in detail. A concise and practical outline for the control of fluid equilibrium and restoration of an established imbalance occupies the latter half of the monograph.

In discussing acid-base balance, sulfates and phosphates are described as acid anions. The accumulation of these anions in renal insufficiency or of keto-acids in diabetic coma displaces bicarbonate resulting in acidosis. However, current thought suggests that the accumulation of hydrogen ions in association with the anions which are not actually acidic causes the change in blood pH.

This monograph is of value to the practitioner for its emphasis upon the clinical assessment of patient's history and objective findings. The management of parenteral fluid therapy when full laboratory control is unavailable seems entirely feasible with the methods outlined therein. C. R. SHUMAN

Books received for review by THE AMERICAN JOURNAL OF CLINICAL NUTRITION are acknowledged in this column. As far as practicable those of special interest are selected, as space permits, for a more extensive review.

Polyscience by Frank H. Kelly, Vantage Press, New York, 1958, pp. 145, $2.95.

Low-Fat Cookery by Evelyn S. Stead and Gloria K. Warren, McGraw-Hill, New York, 1956, pp. 184, $3.95.

Home Economics—Careers and Homemaking by Olive A. Hall, Wiley, New York, 1958, pp. 301, $4.25.

Chemistry of Lipides as Related to Atherosclerosis, compiled and edited by Irvine H. Page, Thomas, Springfield, Ill., 1958, pp. 342, $8.50.

Food, Nutrition and Diet Therapy, ed. 2 by Marie V. Krause, Saunders, Philadelphia, 1957, pp. 621, $6.00.

Ciba Foundation Symposium on Chemistry and Biology of Mucopolysaccharides, edited by G. E. W. Wolstenholme and M. O'Connor, Little, Brown, Boston, 1958, pp. 329, $8.50.

Neomycin. Its Nature and Practical Application, edited by Selman A. Waksman, Williams and Wilkins, Baltimore, 1958, pp. 412, $5.00.

General Biochemistry, ed. 2 by Joseph S. Fruton and Sonia Simmonds, Wiley, New York, 1958, pp. 1,077, $18.00.

Die Dystrophie, Spätfolgen und Dauerschäden, Georg Thieme Verlag, Stuttgart, 1958, pp. 202, D.M. 19.60 ($4.65).

The Chemistry and Chemotherapy of Tuberculosis, ed. 3 by Esmond R. Long, Williams and Wilkins, Baltimore, 1958, pp. 450, $12.00.

Annual Review of Medicine (Vol. 9), edited by David A. Rytand, Annual Reviews, Inc., Palo Alto, pp. 530, $7.00.

Care of the Premature Infant by Evelyn C. Lundeen and Ralph H. Kunstadter, Lippincott, Philadelphia, 1958, pp. 367, $8.00.

Clinical Enzymology, edited by Gustav Martin, Little, Brown, Boston, 1958, pp. 241, $6.00.

Abstracts of Current Literature

ITEMS OF GENERAL INTEREST

Abbreviations for Iodinated Amino-Acids and Derivatives from the Thyroid Gland. C. R. Harington, R. Pitt-Rivers, A. Querido, J. Rocke, and A. Taurog. *Nature*, 179: 218, 1957.

A large literature is developing on the use of the thyroid hormones, their precursors and derivatives, and there has been some confusion in the use of symbols and contractions for these substances.

At the Ciba Foundation Colloquium in June, 1956 on the "regulation and mode of action of thyroid hormones" a discussion was held in which agreement was reached on suitable symbols for the iodinated thyronines and hyrosines. The discussion will be published in full but the authors wish to direct the attention of research workers to the following accepted symbols:

Thyroxine	T_4
Thiiodothyronine	T_3
Diiodothyronine	T_2
Monoiodothyronine	T_1
Diiodotyrosine	DIT
Monoiodotyrosine	MIT
Tetraiodothyropyruvic acid	KT_4
Triiodothyropyruvic acid	KT_3
Tetraiodothyroacetic acid	TA_4
Triiodothyroacetic acid	TA_3

To distinguish between isomers, the symbol can be preceded by numbers indicating the positions of halogen atoms in the benzene rings of the thyronine; thus, the three diiodothyronines would be 3:5-T_2, 3′:3′-T_2 and 3′:5′-T_2. F. E. HYTTEN

Unilateral Sweating of the Submental Region After Eating. (Chorda Tympani Syndrome.) A. G. Young. *Brit. M. J.* 2: 976, 1956.

A case is described of a 12-year-old girl who had an erythematous reaction accompanied by profuse sweating of the right submental area soon after eating. This occurred with most foods and after sucking cotton wool soaked in 0.5 per cent acetic acid, although drinking tea without food was not effective. The girl had her tonsils and adenoids and tuberculous glands removed from the right side of her neck at the age of two years.

Tincture of belladonna reduced the erythema without affecting the sweating but right lingual nerve (chorda tympani) block abolished both. Stellate ganglion block was without effect. The sparse literature on this subject is reviewed and it is suggested that abnormal regeneration of nerve fibers may follow operations on the neck where the lingual nerve is damaged. Possible lines of treatment are discussed briefly. It is suggested that the syndrome should be called "the chorda tympani syndrome." F. E. HYTTEN

A Simple Aid in Calculation of Diets. R. J. Slonim, Jr. *J.A.M.A.* 162: 1233, 1956.

In this brief paper the author has recommended a simple formula for the calculation of various types of diets. This is based on a number of assumptions, such as that the "ideal" body weight is 110 lb for a person five feet tall, with five lb added for each additional inch, etc. The formula presented and the nomogram are said to save time and effort in preparing the type of diet needed to induce a weight change.

While this is a laudable attempt, it seems to this reviewer that the problem of weight changes rarely follows such mathematical niceties. Instead, like the dose of insulin, therapy must be tailor-made to the individual patient. S. O. WAIFE

Oxygen Consumption in the Rat During Prolonged, Acute Starvation. R. G. Sisson and S. Lang. *Proc. Soc. Exp. Biol. & Med.* 93: 173, 1956.

In order to determine whether or not the decrease in oxygen consumption occurring in starving animals is due to inactivation of the thyroid, rats were surgically thyroidectomized, and others were given triiodothyronine and subsequently starved. Oxygen consumption measured daily for ten days was highest in the hyperthyroid and lowest in the hypothyroid group, but showed a similar decline with time in all starved groups.

M. SILBERBERG

Use of d-Amphetamine to Curb the Increased Appetite and Over-Eating Induced by Reserpine Therapy. S. Kinard, L. C. Mills, J. Terrell, and J. H. Moyer. *J. Amer. Geriatrics Soc.* 4: 1073, 1956.

The authors report on the use of d-amphetamine to curb the increased appetite and over-eating induced by reserpine therapy. They report on 47 patients treated with reserpine alone, and 31 patients treated with reserpine plus d-amphetamine over a 16-week period. Sixty per cent of the first group gained weight while 97 per cent of the latter group lost weight. The authors point out that d-amphetamine did not have any adverse effect on the hypertension in the obese group.

This study like so many studies on appetite-depressing drugs is unimpressive because of the short duration of the study. To be significant a study of this type should be carried out for at least one year.

K. R. CRISPELL

The Influence of Breast Feeding on Weight Gain in Infants in the First Year. J. Mills. *J. Pediat.* 48: 770, 1956.

Three groups of infants of different ethnic origin and economic circumstances were studied: poor Indian, poor Chinese, and wealthier Chinese. Selection of infants was based on the following criteria: both parents of pure ancestry, infants born to women confined in same hospital, only single births which were physically mature and without congenital anomalies, infants domiciled in the urban area to permit regular home visits and accurate records. Families within each group were of similar socio-economic level. All babies were weighed by the same personnel under completely standardized conditions, at intervals of two weeks for the first twelve weeks and of four weeks until the end of the first year. There were approximately 2,000 home visits to each group. The report is limited to infants with complete records from birth to end of first year (approximately 50 male and 50 female infants in each group). Breast feeding results in a greater weight gain at 24 weeks for poor Indian and Chinese babies but no significant difference was found for wealthier Chinese babies. The weaning diet of the wealthier babies was more adequate and the standard of hygiene was higher, and therefore the artificially fed babies were at no disadvantage. The poor babies received an inadequate diet after weaning, and consequently at twelve months the benefits resulting from breast feeding were no longer obvious.

T. C. PANOS

The Influence of Vitamin Supplementation of the Diets of Pregnant and Lactating Women on the Intelligence of Their Offspring. R. F. Harrell, E. R. Woodyard, and A. I. Graves. *Metabolism* 5: 555, 1956.

Observations on retarded children have shown a high incidence of faulty nutrition in the prenatal history of these mentally defectives. The effect of vitamin supplementation using a double-blind technic was studied in 2,400 women in two maternity clinics, one in Norfolk, Virginia, and one in Kentucky. Intelligence testing of their offspring at ages three and four years was conducted using conventional testing methods. The mean intelligence of children born of 612 women in Norfolk was significantly higher in those whose mothers had been given vitamin supplements during the later part of pregnancy than in those whose mothers received an inert control tablet. The benefit of supplementation was most apparent in those receiving only thiamine or only ascorbic acid. The mean duration of antepartum supplementation in Norfolk was 134 days. In the Kentucky study no significant differences were noted between supplemented and unsupplemented groups. It was found that the usual diet consumed by the Kentucky families was more nearly adequate than that used in the Norfolk homes. The results of this study indicate that dietary improvement through vitamin supplementation may be of importance in the development of the central nervous system during the prenatal period although the limitations of the observations do not permit final conclusions to be drawn.

C. R. SHUMAN

Serum Enzymes I. Serum Lactic Dehydrogenase in Myocardial Infarction. L. P. White. *New England J. Med.* 255: 984, 1956.

When there is necrosis of heart muscle, such as is found in myocardial infarction, significant amounts of glutamic oxalacetic transaminase are released into the blood serum. This method has been used in the diagnosis of myocardial infarction but there have been three main drawbacks to the use of this technic. The high levels of this enzyme are seen only 24 to 72 hours after the infarction occurs. Some patients with myocardial infarctions do not show a high concentration of this enzyme. The method for determination of this enzyme is laborious. Since the heart is rich in enzymes which are probably also released into the blood after infarction, the author of this paper studied the effect of myocardial infarction on levels of serum glutamic oxalacetic transaminase, lactic dehydrogenase, aldolase and hexose isomerase in 50 patients.

The serum level of lactic dehydrogenase was found to be the best index of myocardial infarction. The level of this enzyme was found to be elevated for eight days, while the levels of the other enzymes studied went back to normal two to three days after the infarction. The assay for this enzyme is comparatively simple compared to the determination of glucose oxalacetic transaminase.

Increased levels of all the enzymes are found when there is tissue damage. This can occur in the liver, skeletal muscle, kidneys or brain as well as the heart, so the test is not specific. Abnormal levels of these enzymes therefore have to be interpreted with regard for the clinical situation. M. W. BATES

Serum Enzymes in Muscular Dystrophy and Certain Other Muscular and Neuromuscular Diseases. I. Serum Glutamic Oxalacetic Transaminase. C. M. Pearson. *New Eng. J. Med.* 256: 1069, 1957.

A transitory increase in the serum level of glutamic oxalacetic transaminase occurs after acute myocardial injury. Since skeletal muscle cells are also rich in this enzyme a study was made of the effect of muscular dystrophy and other muscular diseases on the serum level of glutamic oxalacetic transaminase.

Eighty-seven cases of progressive muscular dystrophy were studied. While 90 per cent of the muscular dystrophy patients 18 years of age or younger had elevated levels of glutamic oxalacetic transaminase, only 15 per cent of the patients 18 years of age or older had elevated levels. No significant correlation was found to exist between the duration of the disease and the transaminase level. The highest levels were found in the intermediate stages of the disease; the lowest, in the very mild or very severe cases. The enzyme levels seemed to be higher in the cases in which the disease progressed more rapidly. In pseudohypertrophy there is an increased enzyme level. In active, progressive stages of muscular dystrophy the serum transaminase levels may remain continuously elevated for many months or several years.

The serum transaminase level was also measured in several miscellaneous types of primary muscle diseases and neuromuscular atrophies. Normal or slightly elevated values were found except in cases of acute dermatomyositis and paroxysmal myoglobinuria where there were high levels. The enzyme levels were normal in six children with poliomyelitis where there was progressive muscular atrophy. M. W. BATES

Effect of Vitamin-B Complex on Healthy People in a Warm Climate. E. M. Glaser and B. H. Livett. *Brit. Med. J.* 1: 1331, 1957.

Vitamins of the B complex are commonly prescribed for healthy, well-fed people in the tropics on the grounds that more are needed, but no evidence to support this thesis has been put forward. In the experiment described here, Singapore University students were given pills containing a mixture of B vitamins and identical "dummies." There was no difference in the incidence of illness or in the subjective sensations of well-being between the two groups. Incidentally, serum cholinesterase was estimated in the two groups of students. The level was significantly raised in the group taking the vitamin B preparation; no explanation for this is offered. F. E. HYTTEN

Nutritional Deficiency Diseases Today. W. J. Darby. *J. Am. Dietet. A.* 33: 17, 1957.

In this article, the author briefly reviews our current knowledge of the incidence of nutritional deficiency diseases. It is pointed out that "whereas, in the United States, the incidence of recognizable deficiency states had become infrequent during the past decade, a recent reawakening of interest of nutritionists in occurrence of such states has resulted because of (a) the sudden recognition of new deficiency states, (b) the realization that certain metabolic diseases are influenced by dietary factors previously considered to be of little importance, and (c) the spotlighting of nutritional diseases throughout the world."

Cited as furnishing evidence of new deficiency diseases are studies in which pyridoxine deficiency was produced: (1) experimentally in adult human subjects through administration of vitamin B_6-low diets and desoxypyridoxine, (2) in infants fed a pyridoxine deficient diet or a highly processed infant food, and (3) in isoniazide-treated persons. Understanding of the role of tocopherol in human nutrition is increasing through work with infants and adults with steatorrhea and through studies at the Elgin State Hospital which deal with estimation of body stores of tocopherol and the extent of association of these stores with biochemical or metabolically detectable findings.

Kwashiorkor in Africa, Asia, Central America, South America (and any other areas where high-carbohydrate, low-protein diets are fed in early life;) pellagra in Yugoslavia, Egypt, Basutoland, and Southern Rhodesia; and endemic goiter in Chile, Central America, Panama, and other countries have occasioned awakened interest in diet as a factor in physical and mental health.

Activities of WHO and FAO in providing acceptable, readily available sources of protein and other dietary essentials are reviewed briefly. The importance of understanding cultural patterns if one is to attempt to change food habits is emphasized. Thus the World Health Organization has taken pioneering steps by sending cultural anthropologists into areas to aid in understanding of the role of food in particular societies, and attitudes toward food and toward change in food habits. The author points out that the problem of nutritional deficiency diseases is of much broader significance and greater complexity than was widely appreciated a few years ago and further observes "it seems unlikely that simplification will occur."

J. M. SMITH

New Findings in Nutrition Research. C. G. King. *J. Am. Dietet. A.* 33: 93, 1957.

The author considers the most serious form of malnutrition in this part of the world is associated with eating too many total calories. Classic deficiency diseases have almost vanished from this part of the world though less severe deficiencies and imbalance continue to plague us. In less favored countries severe

protein deficiencies, represented by pellagra, kwashiorkor, and stunting of growth represent serious detriments to health. More studies on protein needs of infants are needed as it hardly can be accepted that an infant requires more protein than that supplied by human milk. The findings of Elvehjem and associates are commended for the insight afforded us. The author feels specific imbalances may be important causative factors in pellagra and kwashiorkor.

Current developments in understanding of the role of cholesterol in atherosclerosis and obesity are reviewed. Among fields awaiting further investigation are (a) the range of tolerable balances between saturated and trans types of fatty acids to linoleic and arachidonic acids which can be consumed isocalorically without consequent elevated serum cholesterol values; (b) the possible effect of intermediates as squalene; (c) the basic question of whether short-term or long-term shifts in concentration of cholesterol furnish a reliable index of origin and cause of atherosclerosis. It now appears reasonably clear that unsaturated fatty acids have a lower tendency to form cholesterol than solid fatty acids. The case for oleic acid is unsolved as yet. The author decries attributing special qualities for control or appetite suppression to special foods as fat, sugar, or protein.

Studies on weight control have shown that emotionally well-balanced persons can cooperate and establish satisfactory weight reduction. A second phase of the new findings points out the early age range at which obesity becomes a pattern of living. There has been evidence that increased physical exercise does not necessarily direct one to a continued excessive use of calories.

Special problems with children continue to be treatment of diabetics and handling of celiacs and persons with cystic fibrosis or galactosemia. Galactosemia is believed to be a metabolic failure whereas the fibrosis is genetic. Celiac disease symptoms seem to result primarily from abnormal metabolism of glutamine.

Fluoridation of water when well handled has resulted in a 50 to 70 per cent decrease in incidence of tooth decay for children whose teeth are in the formative stage. There is additional evidence each year that other mineral elements can give protection beyond that supplied by fluorides and other minerals. Phillips and co-workers report an organic concentrate which exerts a protective effect against dental caries in experimental animals. J. M. SMITH

Studies Concerning the Specificity of the Skeletal Effects of Enriched Diets in Aging Mice. M. Silberberg and R. Silberberg. *Lab. Invest.* 6: 372, 1957.

In weanling male mice of strain C57BL a diet enriched with 25 per cent lard fed *ad libitum* through life accelerated skeletal growth and development. If fed isocaloric quantities of rations enriched with lard, cornstarch or lactalbumin, respectively, this effect was found to be due to the high calorie content of the diet as well as to the lard specifically. The present investigation was undertaken in order to determine whether or not after consumption of high-fat diets similar principles apply to the promotion of skeletal aging and the development of degenerative joint disease. Weanling male mice of strain C57BL were housed individually and fed throughout life isocaloric amounts of a high-fat (24.5 per cent), high-carbohydrate (65.1 per cent), or high-protein (55.7 per cent) diet, respectively. The rations were adequate in vitamin and mineral requirements. The epiphyseal growth zones and the knee joints were studied microscopically and the findings correlated to the weights and caloric intake of the animals. Epiphyseal aging and the evolution of osteoarthritis were promoted by feeding the high-fat diet as compared with the animals fed the high-protein diet. The mice fed the high-carbohydrate diet took an intermediate place between the other two rations. Single housing of the animals decreased food consumption and by producing a relative state of undernourishment delayed, as compared to controls, epiphyseal aging and the evolution of osteoarthritis. M. SILBERBERG

Lesions in "Yellow" Mice Fed Stock, High-Fat, or High-Carbohydrate Diets. R. Silberberg and M. Silberberg. *Yale J. Biol. & Med.* 29: 525, 1957.

"Yellow" mice such as those of strain YBR/Wi are represented by two genotypes which differ in regard to one gene. Individuals possessing the Y′ gene are yellow-coated and become obese when fed enriched diets; individuals not possessing this gene are gray-coated and do not become obese when fed such diets. Male and female mice of strain YBR/Wi, yellow-coated as well as gray-coated, were fed *ad libitum* stock, high-fat or high-carbohydrate diets, respectively, and the visceral lesions in these animals were recorded. In obese as well as nonobese mice squamous metaplasia of the respiratory and urinary bladder epithelia, of the epithelia of the pancreatic ducts and of the endometrial glands were observed. In association with the coexisting keratomalacia and faulty skeletal molding noted in these mice, the epithelial metaplasia is probably due to disturbances in the metabolism of vitamin A possibly with involvement of the thyroid gland. Pyelonephritis and amyloidosis were common findings. Amyloidosis was attenuated by consumption of the high-fat diet. Aortitis and myocarditis were frequent and obviously due to the lowered resistance of these animals to infection. Hyperplasia and hypertrophy of the islets of Langerhans were particularly frequent after consumption of the high-carbohydrate diet. M. SILBERBERG

Metabolism of the Essential Fatty Acids. II. The Metabolism of Stearate, Oleate, and Linoleate by Fat-Deficient and Normal Mice. J. F. Mead, W. H. Slaton, Jr., and A. B. Decker. *J. Biol. Chem.* 218: 401, 1956.

Doses of C^{14}-labeled methyl esters diluted with corn oil were administered orally to fat-deficient (male mice

which had been on a fat-free diet three months from weaning) and normal mice, and oxidation rates were determined by measuring respiratory CO_2 at varying intervals. The total lipids from pooled carcasses were analyzed for activities in the saturated and unsaturated fatty-acid fractions.

Stearate and linoleate were oxidized to a significantly greater extent by fat-deficient than by normal mice, while for oleate the reverse was true. Essential fatty-acid deficiency does not appear to decrease the ability of the animal to catabolyze fat. The discrepancy in the case of oleic acid cannot be explained. The incorporation of C^{14} into cholesterol (as compared to that of total fat) appeared to bear an inverse relationship to that of total respiratory CO_2.

The condition of fat deficiency does not seem to hinder either hydrogenation of unsaturated or the dehydrogenation of saturated acids as shown by activity in unsaturated acids from stearate fed animals and of the saturated acids from those fed oleate and linoleate. The metabolic pathway of linoleic acid was studied but will be reported elsewhere. M. K. HORWITT

A Study on the Narcotic Action of the Short Chain Fatty Acids. F. E. Samson, Jr., N. Dahl, and D. R. Dahl. *J. Clin. Investigation* 35: 1291, 1956.

Short chain fatty acids are known to have an inhibitory action on many metabolic reactions *in vitro*. Their effects in intact animals, however, have been studied to a very limited extent. In this report, short chain fatty acids (acetate, propionate, butyrate, valerate, caproate, heptanoate, octanoate, polargonate, and caprate) were administered intraperitoneally or intravenously as the sodium salt into rats, frogs, chicks, mice, dogs, and guinea pigs. Unconsciousness was produced promptly in the experimental animals. The amount of fatty acid which produces this response decreases with the increase in chain length. The introduction of a hydroxy group into the carbon chain reduces the narcotic action. Hemolysis was not produced. The narcotic action here would seem to be a direct effect of the fatty acid anion on central nervous tissue. It is likely that the fatty acid salts inhibit metabolic activity of cerebral tissue as they do in muscle and yeast. S. O. WAIFE

GASTRIC FUNCTION AND NUTRITION

The problem of pathogenesis of peptic ulcer has not been solved although it is apparent that the secretion of acid and pepsin is essential for the development of this disease. Psychodynamic forces may precipitate ulcer formation or may aggravate an extant lesion; however, it must not be assumed that psychologic factors represent the basic etiology of this disease at our present state of knowledge.

Comparison of Gastric Emptying and Secretion in Men and Women with Reference to Prevalence of Duodenal Ulcer in each Sex. M. Booth, J. N. Hunt, J. M. Miles, and F. A. Murray. *Lancet* 1: 657, 1957.

Since duodenal ulcer is more common in males than females one might expect differences between the sexes in gastric emptying time and secretion. This was put to the test in 20 young male students and 19 young female students by means of saline and water test meals.

There was no sex difference in the gastric emptying time but the mean secretion of acid, chloride, and pepsin was about one and one-half times greater in men than in women. It is tentatively concluded that the sex difference in the incidence of duodenal ulcer does not depend on differences in gastric emptying, although it might be related to differences in secretion. F. E. HYTTEN

Influence of Hydrochloric Acid on Gastric Secretion and Emptying in Patients with Duodenal Ulcer. J. N. Hunt. *Brit. M. J.* 1: 681, 1957.

One theory of the gastric hyperfunction occurring in patients suffering from duodenal ulcer is that there is a failure of the duodenal mechanisms which inhibit gastric secretion and emptying. In the experiments reported here test meals of 750 ml of water containing 75 g of glucose with or without 20 meq HCl/liter of meal were given to 27 volunteers in good health and 16 patients suffering from duodenal ulcer.

There was no evidence of an abnormally rapid gastric emptying time in the subjects with duodenal ulcers, whereas in the others, the less the secretion of acid in response to the test meal the more effective was the added acid in showing the emptying.

The 16 patients with ulcers had about twice the mean normal outputs of acid, chloride, and pepsin in response to the control meal; the added acid reduced the output of acid in ten of these patients and augmented it in the other six. In the healthy subjects the mean acid output was reduced by the added acid. The findings are discussed and it is concluded that the evidence does not support the theory of a failure of the duodenal inhibitory mechanism. F. E. HYTTEN

Inhibition of Gastric Emptying and Secretion in Patients with Duodenal Ulcer. J. N. Hunt. *Lancet* 1: 132, 1957.

It is well known that patients with duodenal ulcer have a gastric hypersecretion of acid and pepsin, and it is traditionally accepted that the gastric emptying is unduly rapid. These features have been tested by means of saline and glucose test meals in 27 healthy persons and 16 male patients with duodenal ulcer. The author has shown previously that a saline meal has minimal effect on the duodenal osmoreceptors which actively inhibits gastric emptying, while a solution of 10 per cent glucose has a powerful effect.

There was no convincing evidence of unduly rapid emptying of either of these meals in the patients with duodenal ulcer, but the secretory response to the meals,

measured as amounts of acid, chloride, and pepsin, was about twice normal in the persons with ulcer.

F. E. HYTTEN

The basis for treatment of peptic ulcer rests with the provision of a diet which is neither chemically, thermally, or mechanically irritating. Recent controversy concerning the validity of this concept is expressed in the following.

Value of Diet in Treatment of Peptic Ulcer. E. C. Texter, Jr. *Am. J. Digest. Dis.* (n.s.) 2: 130, 1957.

Despite the almost universal use of a standard bland diet in peptic ulcer, evidence for its efficacy as an aid in the healing of active ulcers or in preventing their recurrence is as yet incomplete.

Two studies have recently been carried out, one by Lawrence and another by Doll and associates, in which the therapeutic response of patients with peptic ulcer to a bland diet was compared with a similar group on a near normal diet. No significant differences were observed in the two groups. This is supported by experimental evidence in animals indicating that the type of food administered affects only slightly the level of acidity of the gastric content, and that experimental ulcers heal equally as well on a rough as on a bland diet. However, the essayist cautions that the general experience with peptic ulcer patients, although not well controlled, indicates that ulcer pain is minimized by bland feedings and should not be ignored. He advises its continued use until additional information is available.

One supporting bit of evidence was that obtained in excisional ulcers containing a silk suture at the base, which tended to become chronic if the animals were fed a rough diet. These ulcers healed more promptly when a bland diet was instituted. J. B. HAMMOND

The Effect of Spice Ingestion Upon the Stomach. M. A. Schneider, V. DeLuca, Jr., and S. J. Gray. *Am. J. Gastroenterol.* 26: 722, 1956.

The authors have reviewed and have added studies of their own on the effect of spices on gastric secretion and on patients with gastrointestinal disorders.

Fifty patients with active duodenal or gastric ulcer with demonstrable craters by x-ray were maintained on a routine ulcer regime including a progressive "ulcer diet" with interval feedings, antispasmodics, and antacids. In addition, each patient was given one capsule three times daily with meals containing a spice in an amount equivalent to the concentrations used in highly spiced recipes. Five patients in this group developed symptoms during the administration of spices: two with black pepper, one with chili pepper, one with nutmeg, and one with mustard seed. Severe burning epigastric pain, belching, and nausea were associated with the administration of black pepper in two patients requiring discontinuation of this medication. With the exception of the black pepper, the other spices did not alter the healing time of the peptic ulcer as determined clinically or by repeated x-rays of the stomach.

None of the patients who received cinnamon, allspice, mace, thyme, cloves, carraway seed, paprika, or sage developed symptoms.

Patients with inactive duodenal ulcer who were not on an ulcer regime were given cloves, mustard seed, black pepper, and chili pepper without apparent effect except in two patients. One developed belching after taking cloves, the other developed symptoms after administration of black pepper.

No significant changes were noted in the gastric mucosa of 15 patients following the ingestion of cinnamon, nutmeg, allspice, thyme, chili pepper, cloves, and paprika. Thyme and mustard seed produced a mild erythema but no symptoms. Chili pepper produced a severe hyperemia without symptoms. The uropepsin excretion remained essentially unchanged after 21 to 55 days during the administration of cinnamon, nutmeg, allspice, and cloves. One patient who had pre-existing hypertrophic gastritis of the antrum developed a marked aggravation of the inflammatory changes as observed gastroscopically following the administration of black pepper.

The authors concluded that, although the spices did not stimulate gastric acid secretion, black pepper, chili pepper, cloves, mustard seed, and probably nutmeg should be considered gastric irritants and it is implied that these should be omitted from the diet of patients with ulcerative or inflammatory changes of the gastric mucosa. J. B. HAMMOND

A Long-acting Inhibitor of Gastric Secretion. A. H. Douthwaite, J. N. Hunt, and I. MacDonald. *Brit. M. J.* 2: 275, 1957.

This is a preliminary report on (1-methyl-2-pyrrolidyl) methyl benzilate methyl methosulphate (IS 499), a substance with atropine-like properties. A total of 98 test meals were conducted for seven normal subjects to test the effect of the drug on gastric secretion and a number of tests were also made on patients with gastric or duodenal ulcers.

In 8 of the 12 subjects the acid secretion was reduced by 40 to 60 per cent three hours after a 5 mg dose of IS 499 and in 6 of 12 subjects a similar reduction was found nine hours after an 8 to 10 mg dose. There were no troublesome side effects after doses of this size although gastric emptying was somewhat slowed.

F. E. HYTTEN

The postoperative effects of subtotal gastrectomy and gastroenterostomy may have serious nutritional consequences in about ten per cent of patients. Loss of weight due to impaired absorption and "dumping" account for most of the difficulties encountered in this group.

A Study of Motility in the Gastric Remnant Following Subtotal Gastrectomy. G. L. Jordan, Jr., H. L. Bar-

ton, and W. A. Williamson. *Surg. Gynec. and Obst.* 104, 257, 1957.

Subtotal gastrectomy is a common operation for complications of peptic ulcers or gastric cancer. Varying amounts of the stomach are removed, depending, in the case of the benign lesions, principally, on whether or not an associated vagotomy is done. Among the early postoperative problems encountered in patients undergoing subtotal gastrectomy is gastric retention, particularly about eight to ten days after operation. The study reported by Jordan and his associates was undertaken to assess the possibility that alterations in the motility of the gastric remnant are involved in the syndrome of postoperative retention.

Forty-six patients were studied between the ninth and fifteenth postoperative days; 44 were asymptomatic at this time, while two had gastric retention due to roentgenographically demonstrable obstruction in the efferent loop just beyond the gastrojejunal stoma. In most patients (42), 70 to 80 per cent of the stomach had been removed; in no patient was a vagotomy performed, though one patient had had a previous vagotomy and gastroenterotomy. Half of the patients had a Billroth II type procedure, half a Billroth I; most had been operated on because of duodenal ulcers.

Motility of the gastric remnant was estimated by roentgenographic studies and by measurements of intraluminal pressures before and after ingestion of a test meal. When barium was ingested, it flowed rapidly through the stoma. Serial films revealed progressive emptying of the gastric remnant, with a gradual decrease in its apparent size, but in most cases there was no change in contour. In only two patients were definite evidences of weak contractions visualized; in four others, questionable peristalsis was noted; in no patients were strong contractions observed. Similarly, the recordings of intragastric pressure were characterized by a minimum of activity.

These observations indicate that motility in the gastric remnant two weeks after operation is minimal; this behavior is similar to that which occurs in this portion of the intact stomach. Since emptying occurred rapidly from the gastric remnants, lack of gastric peristalsis is not a primary factor in the development of gastric retention in the early postoperative period. The authors feel that the normal tone of the gastric wall, jejunal peristalsis, changes in intra-abdominal pressure, diaphragmatic motion, gravity, and a patent stoma and unobstructed small bowel are the important factors in emptying of the gastric remnant. S. M. Levenson

Comparison of Side-effects After Partial Gastrectomy and Vagotomy and Gastro-enterostomy. H. T. Cox and D. F. Kerr. *Brit. M. J.* 1:1211, 1957.

A comparison is made of the side-effects in 100 patients who had had a partial gastrectomy for duodenal or anastamotic ulcer and in 100 cases of duodenal ulcer treated by vagotomy and posterior gastroenterostomy. "Each type of operation was carried out in comparable and unselected cases."

The results are reported at length; the broad conclusions are as follows: there is no material difference in the incidence or severity of biliary regurgitation after the two operative procedures. Attacks of dizziness, weakness, palpitation, and sweating "dumping" were generally uncommon but the incidence was less after vagotomy and gastroenterostomy. The outstanding advantage of vagotomy and gastroenterostomy over partial gastrectomy is in the nutrition of the subject; more of them were able to take a normal sized meal (77 compared to 55), more took a sweet course with their meal and considerably fewer suffered from selective impairment of digestion of certain foodstuffs. F. E. Hytten

The adrenal steroids have been demonstrated to influence the rate of gastric secretion of acid and pepsin. This finding may be important in the pathogenesis of ulcer formation, particularly under conditions of stress in which stimulation of steroid secretion occurs. Further studies on the propulsive effects of these hormones are in order.

Effects of Adrenocortical Steroids on the Propulsive Motility of Small Intestine. D. Streeten, B. Hirschowitz, K. Henley, and H. Pollard. *Am. J. Physiol.* 189:108, 1957.

Adrenal cortex extract in low concentrations (1:1400–1:250) increases the peristaltic contractions of the small intestine and restores to fatigued intestinal segments normal peristaltic activity and the ability to propel fluid against a pressure gradient, *in vitro* (modified Trendelenburg technic). Adrenal cortex extract in high concentrations (1:150–1:25) reversibly inhibits or abolishes peristalsis *in vitro*. In adrenalectomized rats *in vivo*, adrenal cortex extract increases the rate of propulsion of dyes along the small intestine in moderate doses (1 ml b.i.d.), and decreases propulsion in large doses (5 ml b.i.d.). Cortisone, hydrocortisone, and corticosterone in the amounts present in stimulant doses of adrenal cortex extract had no effect on intestinal propulsion, *in vivo*. Doses of aldosterone (0.1 and 0.5 μg b.i.d.) comparable with the amounts contained in the extract used and large doses of the electrolyte-controlling steroids, desoxycorticosterone (2.5 and 5 mg) and corticosterone (2.5 mg), reproduced the stimulant effects of the extract, *in vivo*. It is possible that the effects of aldosterone may be of significance in controlling intestinal motility under physiologic and some pathologic conditions. Authors

The incidence of gastritis as detected by biopsy in patients with dyspepsia is surprisingly high and may force revision of the current status of this entity. Gastric biopsy by the methods employing suction of mucosa into a tube for excision may induce bleeding and must be performed with care.

Investigation of Non-Ulcer Dyspepsia by Gastric Biopsy. A. Wynn-Williams, F. Edwards, T. H. C. Lewis, and N. F. Coghill. *Brit. M. J.* 1:372, 1957.

Something like one-quarter of the outpatients at medical clinics complain of dyspepsia the first time they attend. Of 775 such cases, 468 were found to have no ascertainable basis for the dyspepsia and 200 of these were investigated by gastric biopsy.

The classification of gastric mucosal lesions is discussed and the results presented in detail. In 45 per cent of the cases examined the mucosa appeared to be normal. There were mild changes, mostly of chronic gastritis, in 36 per cent and more severe changes (superficial gastritis) in 4.5 per cent. In the remaining 14.5 per cent chronic atrophic gastritis was diagnosed. There was no obvious sex difference in the incidence of gastritis but the atrophic type became more common with increasing age. F. E. HYTTEN

Jejunal Biopsy After Partial Gastrectomy. I. McLean Baird and O. C. Dodge. *Quart. J. Med.* n.s. 26; 393, 1957.

Biopsy of the small intestine is now possible with various technics and attempts are being made to relate histologic changes to clinical findings, particularly in malnutrition and intestinal malabsorption. The histologic appearance of "normal" jejunal mucosa, taken from patients during partial gastrectomy for peptic ulcer, is described in this article and the appearances are compared with those found subsequent to this operation. The postoperative specimens were obtained using Wood's gastric biopsy tube and the method is explained.

No histologic changes were seen in the majority of specimens, but hyperemia and edema of villi, villous atrophy or mild inflammatory changes in the stroma were found in the remainder. Despite the relationship between malnutrition and mucosal changes in the gastrointestinal tract, there was no close association between histologic appearances and the incidence of steatorrhea (five patients) or iron deficiency anemia (17 patients), nor were changes related to the time between operation and biopsy.

W. H. J. SUMMERSKILL

Production of Gastric Ulcers in Dogs on Protein Depletion Regime. P. F. Hahn, P. Baugh, and D. L. Foster. *Proc. Soc. Exper. Biol. & Med.* 95:238, 1957.

Dogs were made anemic by bleeding of about one-fourth of the volume of circulating blood on three consecutive days and on the fifth day. Anemia was maintained by bleeding at such intervals as to maintain one-half of the normal hematocrit. Iron was replaced by intravenous injections of saccharized iron oxide but the animals were fed a diet low in protein (7.4 per cent). Many of the dogs thus treated developed acute peptic ulcers with death resulting from perforation of the stomach. Weight loss under the conditions of these experiments was invariably considerable. The authors do not speculate on the relationship of lowered and altered distribution of circulating proteins or depletion of body protein stores in the induction of these gastric ulcers. M. SILBERBERG

Azotemia noted after acute gastrointestinal hemorrhage is generally attributed to the absorption of the nitrogen load from small intestine during the period of reduced renal function secondary to circulatory insufficiency.

Induced Azotemia in Humans Following Massive Protein and Blood Ingestion and the Mechanism of Azotemia in Gastrointestinal Hemorrhage. T. D. Cohn, M. Lane, H. Zucherman, N. Messinger, and A. Griffith. *Am. J. M. Sc.* 231:394, 1956.

Concentrated protein meals and blood meals were fed by nasogastric tube to normal human subjects in order to observe the effect upon blood urea nitrogen and 24-hour total urinary nitrogen excretion. The blood feeding produced a small rise in blood urea nitrogen while the protein feedings (beefsteak or protein concentrate) produced a large rise in blood urea nitrogen. The azotemia appears to be dependent upon the degree of absorption of ingested protein. Apparently the protein of whole blood is not absorbed as readily as the other protein sources studied. The urinary nitrogen excretion is directly proportioned to the degree of absorption of ingested protein. The important factors related to azotemia in gastrointestinal hemorrhage may well be (1) the size of the hemorrhage; (2) reduced blood volume and diminished renal excretory function, (3) pre-existing renal disease; and (4) protein feeding which may serve as a source for an early rise in blood urea nitrogen. C. R. SHUMAN

The Lack of Effect of Ingested Ferrous Sulfate on the Guaiac Test for Occult Blood in the Stool. J. C. Harvey. *Am. J. M. Sc.* 232:17, 1956.

The problem of whether or not ingested iron produces a false positive test for occult blood was studied by performing the Guaiac test upon stools of 116 individuals receiving ferrous sulfate 0.9 g. daily for three days. In addition, some of the subjects were fed meat in order to observe its effect upon the Guaiac test of stools. It was found that the iron salts produced black and tarry stools but failed to produce a positive reaction with the gum Guaiac test for occult blood. The amount of blood contained in the stools of those consuming meat did not produce a positive Guaiac test. C. R. SHUMAN

Metabolic Alterations in Surgical Patients. VIII. Studies Involving Iron and Magnesium Metabolism in Patients with Gastrointestinal drainage. S. Levey, W. E. Abbott, H. Krieger, and J. H. Davis. *J. Lab. & Clin. Med.* 47:437, 1956.

The authors present balance studies on magnesium and iron carried out on four surgical patients. The

patients were in positive iron balance during most of the study. No change in iron balance occurred which could be attributed to operative stress. The amount of iron lost was not dependent on the volume of gastrointestinal drainage, although this route accounted for the largest losses in most of the patients.

The amount of magnesium lost did seem to correlate with the volume of gastric juice. There was some suggestion that the excretion of magnesium was reduced by operative stress. It was possible to maintain a positive magnesium balance by giving 100 mg or more per day. These should be regarded as preliminary observations. The duration of the study is too short to draw any definite conclusions. K. R. CRISPELL

Gastrointestinal Regulation of Water and Its Effect on Food Intake and Rate of Digestion. S. Lepkovsky, R. Lyman, D. Fleming, M. Nagumo, and M. M. Dimick. *Am. J. Physiol.* 188: 327, 1957.

An investigation was undertaken to determine the effects of water deprivation during meals in rats. Food intake, gastrointestinal solids water content, rate of digestion, and tissue water content were studied. Rats fed without water ate less food than rats fed with water. The gastric contents of all animals fed with or without water was approximately 49 per cent water and indicates close regulation of water in the gastric contents. When fed without water, rats regulate their food intake so that it matches the amount of water that they can mobilize from their own tissues thereby maintaining the proper water: food ratio in the gastric contents. How this is reflected in the mechanisms that control food intake is unknown. The water found in the gastrointestinal contents of rats fed without water is furnished by selected tissues, especially the skin, probably the adipose tissues and perhaps other tissues. The contents of the intestinal lumen contains about 76 per cent of water in all of the rats irrespective of the availability of water with meals. The total solids in the intestinal lumen of the rats eating without water averaged 0.39 g and 0.52 g for the rats eating with water. The regulation of both water and solids in the intestinal lumen indicates that it acts as though it were a part of the internal environment. Withholding of water during meals does not appear to interfere with digestion but it definitely decreases appetite and effects a reduction of food intake.

AUTHORS

VITAMIN E

New information concerning vitamin E in human metabolism is slowly evolving as a result of intensive investigations stimulated by the many observations of its physiologic effects upon experimental animals. It is interesting to note that deficiency of vitamin E in monkeys produces a syndrome resembling muscular dystrophy. Increased reactivity of biologic materials from mammals depleted of vitamin E has been observed. An inverse relationship exists between plasma tocopherol and erythrocyte hemolysis in hydrogen peroxide.

Vitamin E Deficiency in the Monkey. I. Muscular Dystrophy, Hematological Change and the Excretion of Urinary Nitrogenous Constituents. J. S. Dinning and P. L. Day. *J. Exp. Pathol.* 105: 395, 1957.

Young male and female rhesus monkeys received a purified diet devoid of vitamin E. After a period of from 167 to 391 days, slowness of movement developed; the animals were unable to right themselves, when placed on the side; there were losses of muscle tissue, in particular of the hind leg, and difficulty in breathing. Excretion of creatine, allantoin, and free amino acids was increased and of creatinine decreased. A macrocytic anemia, granulocytosis, and lymphopenia developed. Alternate feeding of 30 μg of alpha tocopherol acetate and injections of 20 μg of alpha tocopherol phosphate improved the physical conditions and the abnormal biochemical values within two to three days, and after two to three weeks complete recovery took place. The experimentally produced conditions in the primate were thus specifically due to the vitamin E deficiency.

M. SILBERBERG

Brain tissues of animals depleted of vitamin E show a marked increase in oxidation products as compared to vitamin E-supplemented controls. This observation may be related to the increase in nucleic acid synthesis noted in tocopherol-depleted animals.

An Increased Incorporation of P^{32} into Nucleic Acid by Vitamin E-Deficient Rabbits. J. S. Dinning, J. T. Sime and P. L. Day. *J. Biol. Chem.* 222: 215, 1956.

Previous experiments with vitamin E-deficient rabbits revealed a close relationship between vitamin E deficiency and the incorporation of formate or glycine into nucleic acids. This seemed to indicate that one of the main functions of vitamin E is to control nucleic acid turnover.

In the present experiment, vitamin E-deficient rabbits incorporated much more P^{32} into their tissue nucleic acids than did normal animals. This is regarded as additional support for the suggestion that the control of nucleic acid turnover is one of the main metabolic functions of vitamin E. M. K. HORWITT

CALCIUM METABOLISM

The absorption of calcium is reduced by phytic acid phosphorus derived from plant sources in the diet. Oxalates have a similar effect upon calcium absorption. The absorptive process is favored by a calcium phosphorus ratio of 1:1. The well-known observations may account for the findings reported in the following study.

Absorption of Calcium and Strontium from Milk and Non-Milk Diets. F. W. Lengemann, C. L. Comar, and R. H. Wasserman. *J. Nutrition* 61: 571. 1957.

As part of an extended investigation on calcium and strontium metabolism it was possible to obtain quantitative data on the effect of milk upon absorption of these two elements in cattle, rats, rabbits and human subjects.

The availability of calcium from milk was contrasted with that from $CaCl_2$ in rats, rabbits and human subjects, and with calcium supplied with grain in cattle, rats and rabbits. In cattle, the retention of single oral doses of Ca^{45} and Sr^{89} gave essentially the same results as the conventional determination of calcium balance. Calves on a milk diet absorbed and retained a very high percentage of the calcium present. Similar animals on a hay diet and grain diet showed a much lower absorption and retention. A calf on hay and grain for 30 days showed the typical high calcium utilization when returned to a milk diet. The calcium retention of an 11-year-old cow was improved by the addition of dried skim milk in the ration.

Young and old rats absorbed about one and one-half times as much calcium^{45} from milk as from a solution of $CaCl_2$ or from $CaCl_2$ + grain. Young and old rabbits showed no increased Ca^{45} absorption from milk. Three out of four human subjects absorbed an average of 34 per cent ingested Sr^{85} from $CaCl_2$ as compared with 82 per cent from milk. B. SURE

Bone density studies have been successfully applied to those structures which are accessible without much surrounding soft tissue. This limitation has excluded the spine and pelvic areas from satisfactory roentgen evaluation of bone density until considerable loss of calcium has occurred.

Influence of Mineral Intake on Bone Density in Humans and in Rats. D. E. Williams, B. B. McDonald, E. Morrell, F. A. Schofield, and F. L. MacLeod. *J. Nutrition* 61: 489, 1957.

For the past 20 years indices to mineral status have been obtained by methods which have evaluated bone development and density. Previous work has been concerned with perfecting technics and instrumentation and in measuring bone density of subjects on self-selected or institutional diets; data to substantiate the reliability of these methods are meager.

Bone densities of young human adults showed variations which appeared to indicate the calcium status of the individual. Characteristic bone densities in young adults were not readily altered by marked temporary changes in calcium intake whereas calcium balances reflected such differences.

Mature rats showed no significant changes in bone density but a small and significant difference in calcium content with increasing intakes ranging from 0.1 to 0.5 per cent of calcium. Young growing rats showed significant increases both in bone density and body calcium with increased intake ranging from 0.1 to 0.5 per cent calcium. Specific activities decreased with increased calcium intakes for mature rats and for growing rats on controlled food intakes. The decrease in activity was interpreted as an indication of increase in calcium reserves. B. SURE

The urinary threshold for calcium has been estimated at 7.0 μμg per 100 ml. Because of the protein binding of approximately 50 per cent of calcium in plasma the available calcium for renal excretion is below the threshold level under usual dietary conditions. With an elevated absorption of calcium the rate of urinary excretion is raised.

The Renal Tubular Reabsorption and Urinary Excretion of Calcium by the Dog. P. P. Poulos. *J. Lab. & Clin. Med.* 49: 253, 1957.

The author first presents his method of determining the diffusible fraction of serum calcium. If this proves to be a reliable method which can be used in most laboratories it will be a major advance in methodology.

The results of studies on the renal tubular reabsorption and the urinary excretion of calcium by the dog are presented. The author points out: "in order to calculate the quantity of calcium which enters the glomerular filtrate per unit of time, one must measure not only the rate of formation of filtrate and the plasma concentration but also the fraction of calcium which is freely diffusible, i.e., filterable."

The excretion and reabsorption of calcium were studied in the dog over a range of plasma concentration from 8 to 37 mg/100 ml. It was found that more than 99 per cent of filtered calcium is reabsorbed by the renal tubules.

The experiments were complicated by the fact that the infusions of calcium chloride or gluconate in amounts necessary to attain high plasma concentrations caused a sharp fall in glomerular filtration rate and led to oliguria.

This is an important study as it may provide a technic to study the effect of various substances on the renal handling of calcium. K. R. CRISPELL

Cortisone has been shown to enhance the absorption of calcium from the intestinal tract. The mechanism whereby the effect is achieved is not clear. The following report demonstrates this action despite the continued high fecal fat content during cortisone therapy for sprue.

A Metabolic Study Following Oral Calcium^{45} Administration in a Patient with Non-tropical Sprue. S. Fink and D. Laszlo. *Gastroenterology* 32: 689, 1957.

The calcium metabolism of a patient with idiopathic steatorrhea was studied by the balance technic employing radiocalcium. During the first half of the 60-day study, the patient received cortisone and was in remission. During this period utilization of ingested calcium gluconate was 79.5 per cent and calcium balances were positive even though there was a high fecal

fat, nitrogen, water, electrolyte, and calcium excretion. Following cortisone withdrawal, the patient gradually developed an exacerbation of his disease. The utilization of ingested calcium gluconate decreased to 18 per cent and the calcium balance became markedly negative.

The data were interpreted as indicating that cortisone improves the absorption of dietary calcium in sprue but does not affect the endogenous fecal calcium. Previous studies have demonstrated improvement following the administration of cortisone in the absorption of carbohydrate, protein, fat, and calcium of patients with idiopathic steatorrhea. This study utilizes the method by which dietary and endogenous calcium absorption could be studied separately and shows that the enhanced absorption of calcium due to cortisone occurred only in dietary calcium. J. B. HAMMOND

The classic treatment for osteoporosis of any cause is testosterone and estrogen in appropriate schedules. Removal of or treatment of the underlying causative factors is possible in certain cases such as those associated with thyrotoxicosis, hyperadrenocorticism, diabetes, etc.

Steroid Therapy for Osteoporosis. E. C. Reifenstein, Jr. *Geriatrics* 12: 139, 1957.

The author poses and answers a series of questions regarding steroid therapy for osteoporosis. This is a rather difficult way in which to present all the facts. He states that the administration of combined hormones has been facilitated by the development of long acting esters for intramuscular injection. No evidence is presented that intramuscular injection has any advantage over oral administration of the hormones. The advisability of continuous versus intermittent administration of estrogens to a female with an intact uterus is not discussed.

The author apparently feels that a combination of estrogens and androgens is the best treatment for osteoporosis. His evidence is based on the fact that steroids cause a retention of calcium even though this disease is thought to be a disorder of bone matrix rather than one of calcium metabolism. Until serial bone biopsies are available the value of steroids in the treatment of osteoporosis remains to be definitely proven.

This form of presentation should not be encouraged unless all possible questions are posed and answered. K. R. CRISPELL

Panel Discussion on Osteoporosis. Moderator: E. Short. Panelists: D. Laszo, N. W. Shock, and G. D. Whedon. *J. Am. Geriatrics Soc.* 5: 363, 1957.

This is an excellent review by well-informed physicians on the problem of osteoporosis. Dr. Laszlo defines the problems associated with the diagnosis of this condition. He presents studies which he has used in an attempt to make an earlier diagnosis and to uncover the basic defect. His studies suggest that patients with osteoporosis absorb calcium from the intestinal tract less efficiently than normal persons. Dr. Whedon discusses the effect of immobilization on calcium metabolism and measures which may be used to combat the adverse effects. The site of involvement of osteoporosis associated with immobilization is different from that of the post-menopausal or senile type in that in the former the distal ends of the bones of the lower extremities are involved first. Dr. Shock discusses the physiology of aging as related to the problem of senile osteoporosis. He discusses specifically the problem of an inadequate diet in the aging population. Finally, Dr. Shorr discusses the management of osteoporosis using hormonal therapy and also the use of strontium.

It is of interest that Dr. Albright has stated that osteoporosis is a disorder of bone matrix rather than of calcium metabolism *per se*. This discussion is centered about calcium metabolism rather than bone metabolism pointing out how little is really known about this prevalent and disabling disease. K. R. CRISPELL

Deficiency of vitamin D or of calcium in the diet results in osteomalacia in adults and rickets in infants and children. The actions of vitamin D in correcting these conditions are still unknown. In addition to an effect upon calcium absorption, there are direct influences upon phosphate metabolism concerned with transfer of these ionic groups across cellular membranes.

Bone Salts Metabolism in Human Rickets Studied with Radioactive Phosphorus. G. C. H. Bauer, A. Carlsson, and B. Lindquist. *Metabolism* 5: 573, 1956.

Rickets is characterized by low serum levels of phosphorus and/or calcium with a poorly calcified skeleton. These abnormalities have been attributed to defective mineral absorption from the gut. The balance studies on which this concept is based are of limited value because they represent the result of two processes: (1) accretion (formation) of bone salt and (2) resorption of bone salt. In order to examine the rate of bone salt formation, $P^{32}O_4$ was administered intramuscularly to rachitic and normal children following which blood and urine samples and bone biopsies were obtained. In children with deficiency rickets, the accretion rate of bone salt was lower than in normal children. The administration of vitamin D restored the accretion rate to normal. In vitamin D-resistant rickets the accretion rate was normal and after massive doses of vitamin D, the rate rose to values higher than those of normal children. The phosphorus of the newly formed bone salts was found to arise from two sources upon treatment with vitamin D: (1) the increased retention of dietary phosphorus and (2) mobilization of phosphorus from other parts of the skeleton. The authors conclude that vitamin D in doses sufficient to cure rickets may cause increased mobilization of bone salts. C. R. SHUMAN

Osteomalacias of Supply, Elimination and of Osseous Origin. A. Lichtwitz, R. Parlier, and B. Hioxo. *Sem. Hôp. Paris* 31: 846, 1955.

Osteomalacias of supply are characterized by (a) hypocalciuria, (b) a paradoxical influence of vitamin D which produces hypocalciuria instead of increasing the urinary calcium as in a normal subject, and (c) by the absence of hypercalciuria in the induced hypercalciuria test (intravenous injection of calcium).

The authors distinguish three varieties of osteomalacia: that due to qualitative alterations of the organic stroma of the bone; that due to a phospho-calcium deficiency; and the variety due to excess elimination.

H. GOUNELLE

The occurrence of hypocalcemia in premature infants has not been adequately studied. Similar observations have been recorded in the newborn infants of diabetic mothers.

Hypocalcemia Occurring on the First Day of Life in Mature and Premature Infants. J. F. Gittelman, J. B. Puncus, E. Schwerzler, and M. Saeto. *Pediatrics* 18: 721, 1956.

Concentrations of calcium, inorganic phosphorus and total proteins in serum or plasma were measured on the first day of life in 838 full-term infants delivered vaginally, 70 infants delivered by Cesarean section and 111 premature infants. Hypocalcemia was defined as a concentration of calcium less than 8 mg/100 ml. Of the 824 full-term babies born to mothers with normal pregnancy and labor, the incidence of hypocalcemia was 1.2 per cent; of the 14 full-term but with abnormal pregnancy and/or labor, the incidence was 100 per cent. Among infants delivered by Cesarean section done at term, the incidence in the group performed because of cephalopelvic disproportion was 13.7 per cent, whereas the incidence among those with abnormal pregnancy was 36.8 per cent. The incidence of hypocalcemia among premature infants was about 50 per cent regardless of the manner or condition of labor and delivery. Mature infants born to mothers who had abnormal pregnancies and/or labor also had a significantly higher mean concentration of phosphorus than those born to mothers who had normal pregnancies and labors. There were no significant differences in mean phosphorus levels among the other births.

Possible causes of the noted hypocalcemia are discussed. The concentration of calcium in maternal serum does not correlate with that of infants' serum and does not appear to be causally involved. The correlation between abnormal pregnancy and/or labor and hypocalcemia in term infants could be partially explained by the phosphate-loading effect of increased destruction of body protein which is said to occur under these circumstances. Also, the adrenocorticosteroids exert a depressive influence on concentration of serum calcium. These steroids are known to be increased during pregnancy and are naturally produced in greatest quantity during the last trimester. It is possible that this factor may be important, particularly in hypocalcemia consistently noted in premature infants. No correlation was observed between the concentrations of calcium and proteins in their plasma. However, a positive significant correlation was observed between body weight and the mean content of calcium in the plasma.

T. C. PANOS

Idiopathic Hypercalciuria. H. Klotz, A. Cohen, and M. Boury. *Sem. Hôp. Paris* 31: 3,434, 1955.

Hypercalciuria occurs when the rate of urinary excretion exceeds 300 mg. The cases observed by the author, accompanied by normal calciuria, seem to be due to a disorder in the tubular reabsorption of calcium. Some believe this disorder is caused by an unrecognized staphylococcus urinary infection. Opinions differ on this point. However, this hypercalciuria responds to treatment with thyroxine and testosterone. Also, hypercalciuria brings about renal lithiasis, cataract, and affects the osseous system and neuromuscular excitability.

H. GOUNELLE

Sialoadenectomy and the Metabolism of Calcium-45 in Bone and Soft Tissues of the Mouse. A. W. Wase and Y. S. L. Feng. *Nature* 178: 1229, 1956.

For a number of years surgical extirpation of the major salivary glands in young experimental rodents has been known to result in a reduced rate of growth and slightly subnormal adult body weights. In the present study, the ability of the sialoadenectomized adult male mouse to metabolize the radioactive isotopes, calcium^{45} and strontium^{89}, was determined. Two hours after administration of either isotope, the mice were killed and the tissues prepared and assayed for isotope content. The calcium^{45} incorporation into the femur of sialoadenectomized rats was 1,106 counts/min/mg of tissue in comparison to 675 counts/min/mg for intact controls. Calcium^{45} incorporation into the thyroid, adrenal, skeletal muscle, heart muscle, spleen, kidney, testes, brain and liver of the sialoadenectomized mice was somewhat higher than the incorporation into these tissues in normal mice. Calcium^{45} incorporation into lung was practically the same for intact and operated mice while the incorporation into thymus was higher in intact than into operated mice. Strontium^{89} incorporation into sialoadenectomized femurs was 175 per cent of control values. These findings were indicative of mineral depletion in the sialoadenectomized mice. Unfortunately the data were only expressed in terms of counts/min/mg with no reference to the total amount of calcium or strontium in the tissues, which is a much more informative measure of the pertaining metabolic state.

A three-day calcium balance study was conducted with intact and sialoadenectomized mice. A higher fecal output and a lower urinary output was noted among the operated animals. The intact mice retained an average of 105.6 mg of calcium per kg of body weight per day in contrast to an average of 69.4 mg/day for sialoadenectomized mice. These values seem to be high for adult mice that would be expected to be approximately in balance. The reasons for the difference in calcium^{45} and strontium^{89} uptake betweeen intact and operated mice and for the difference in degree of positive calcium balance are presently unknown but a source of real speculation as to the mechanism.

J. H. SHAW